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SEQUELAE OF REARING HABROBRACON ON RADIOACTIVE HOST LARVAE[1, 2]

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INTRODUCTION

The ectoparasitic wasp known in genetic literature as *Habrobracon juglandis* (Ashmead) is especially favorable for investigations of radiation effects because of a regularly occurring haploid type, the parthenogenetically produced males, as contrasted to biparental diploid types. The successful induction of lethality in offspring of adult females fed radiophosphorus in honey mixtures (Grosch and Sullivan, 1952) and of fathers similarly fed (in manuscript), has led us to devise a means of feeding radioactive material to larvae.

Radioactive media have been employed in the rearing of Drosophila for investigations of developmental (Arnason, Irwin and Spinks, 1949) and genetic effects (King, 1953). However the present paper reports the initial use of radioactive food in rearing parasitic larvae for comparable studies, achieved by the resolution of a major technical difficulty: The highly evolved larval parasite will not feed except through a break gnawed in the body wall of a natural host. Mashed host caterpillars or caterpillar juice either unadulterated or incorporated into a medium is not acceptable.

Furthermore, although radiophosphorus can pass unchanged from the digestive tract into the tissues of an organism, production of radioactive hosts by growing them on radioactive food is not feasible when, as with Ephestia, development to usable size takes more than twice as long as the half life of P^{32}. Any attempt to set up quantitative ex-

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periments at appreciable levels of radioactivity would prove quite complicated. Also radiological safety might become a prohibitive problem.

To bypass the hazards, indefiniteness and limitations of rearing Lepidopteran host caterpillars on radioactive meal, we have taken a cue from the mother wasp who injects material into the host on which her offspring feed. The exploratory phase of investigation with this new technique is presented below. The variability encountered with attempted quantitation is demonstrated and features of the technique which require refinement are made apparent. Nevertheless, structural aberration and the relative radioresistance of diploids, as well as lethality and developmental delay are clearly shown in rough quantitative relationship to dosage.

Materials and Methods

The Habrobracon stock used was no. 33 wild type, well known for almost 20 years for its high viability and productivity. Groups of "nurse" wasps maintained only for their stinging ability were used to paralyze larvae of the standard host *Ephestia kuehniella* Zeller. The toxin introduced by the braconid sting is a very effective immobilizer of Lepidopteran larvae; yet although locomotor activity ceases, heart action continues at a normal rate (Beard, 1952). The latter feature is fortunate in promoting distribution of agents introduced to the physiological interior of the host larvae.

Paralyzed hosts obtained from "nurse" wasps were checked under the microscope to assure absence of eggs. Then a proportion of these host larvae were injected with 0.04cc of experimental or control fluid using glass capillary needles. Both injected and non-injected hosts were each given five Habrobracon eggs by needle transfer. The sources of these eggs are detailed below. All hosts were arranged in spoke fashion on paper discs contained in petri dishes. During subsequent incubation at 30°C, records were taken of the number of larvae, pupae, and adults obtained. The adults were examined for gross structural abnormality. Unemerged wasps were dissected from their cocoons and the degree and condition of development determined. Longevity records were kept for male adults under starvation conditions.

Eggs from virgin females were used as more homogeneous material than a composite sample of sex-types during a period when we sought the lethal level of radioactivity. Later, as given below, samples from

mated females were used which included fertilized eggs. As indicated in Table 1, records were obtained for haploid males reared on hosts carrying 48 levels of radioactivity determined by injections from solutions containing 125 to 2000 μc/cc. Typically a prepared dilution of radiophosphorus was used as a stock solution for injection on a series of successive days so that the rate of decay has determined the intervals between many of the levels investigated. For this reason the figures are drawn with the dosage decreasing from left to right and a semilogarithmic plot is employed. Between the levels of 125 and 816 μc/cc of injectant, the stock solutions were tinted with neutral red considered to be one of the least toxic of the vital dyes (Koehring, 1931; Sullivan and Grosch, 1953). Injected control experiments were run simultaneously using dye alone. The colored fluid, helpful in developing technique, was dropped as we gained dexterity in manipulation and confidence in our methods. Scattered references to the possible mutagenicity of various dyes (see Clark, 1953) suggest that the decision was a wise one. The junior author is currently checking the possibilities of neutral red mutagenicity in Habrobracon.

At higher levels, experiments were run simultaneously with (a) distilled water and (b) insect Ringer's solution as the diluent for injected radiophosphorus. These results have been pooled since no significant differences were obtained.

Additional experiments with eggs from mated females are not shown in Table 1. One hundred eggs so derived along with an equal number on control caterpillars were used at each level of radioactivity of the injectant above 1050 μc/cc. If the eggs used in this phase of the work are added to the unfertilized eggs used in each category shown in Table 1, the total was 30,560 eggs. For a survey of genetic effects, samples of males from radioactive hosts were mated to untreated females and the egg production of female offspring determined over a ten day period. After incubation the hatchability of the deposited eggs was obtained. The limit placed on the period of observation concerns the important first third of the egg production pattern; i.e., the period known to be of most importance in hatchability studies (Grosch and Sullivan, 1952).

Results

In comparing the hatchability of eggs in radioactive hosts with that of eggs on non-radioactive hosts (Table 1) there is little evidence of

TABLE 1

Levels of radioactivity of stock dilutions used for injection along with sample size and hatchability results of the eggs placed on radioactive hosts.

		Per Cent of Eggs Hatching			
		On Injected Host Larvae			
Injectant µc/cc	Eggs used in each category	Dye plus Radioisotope	Radioisotope in Ringers or Water	Dye Alone	On Uninjected Host Larvae
103	100	95.0		93.0	96.0
108	100	95.0		97.0	96.0
113	100	86.0		94.0	96.0
119	100	90.0		88.0	96.0
125	100	71.0		75.0	82.0
232	150	96.7		99.3	98.7
244	150	98.7		97.3	99.3
256	150	94.7		96.7	97.3
268	150	96.7		96.0	99.3
286	150	96.7		94.7	98.0
312	150	97.4		97.4	99.4
328	450	89.6		96.0	98.4
344	450	93.7		96.4	98.9
360	450	93.3		96.4	98.4
380	435	94.4		94.5	93.3
400	380	89.8		93.2	92.5
498	125	96.8		93.6	95.2
511	250	96.8		99.2	98.4
522	150	94.0		95.3	98.0
536	250	96.0		96.8	99.6
561	300	95.7		97.0	98.3
579	150	90.0		96.7	98.0
593	300	96.7		84.6	97.0
624	280	93.2		93.2	94.6
647	100	87.0		95.0	97.0
669	150	98.0		93.7	99.3
701	150	95.3		96.7	100.0
734	150	89.3		94.7	96.0
775	150	94.7		99.3	99.3
816	150	98.0		96.7	98.7
858	100		98.0		98.0
908	100		100.0		97.0
960	100		98.0		99.0
998	100		97.0		99.0
1050	200		95.5		96.0
1101	100		95.5		96.0
1152	400		97.7		100.0
1216	400		97.7		98.6
1280	350		99.4		99.3
1340	400		98.0		97.6
1420	400		92.7		98.0
1500	400		95.0		96.0
1560	100		93.0		84.0
1640	100		88.0		84.0
1723	400		95.0		95.0
1800	400		95.5		97.6
1900	400		94.3		98.0
2000	300		95.0		91.0
Mean % of Hatchability		93.34 ± 0.98	95.85 ± 1.05	94.58 ± 0.87	96.52 ± 0.57

embryo mortality. There was no correlation between lethal effect and dose, and in many of the higher level experiments, hatchability equaled or exceeded that of eggs on injected hosts. Furthermore, although the hatchability of eggs on radioactive caterpillars is lower than that of eggs on non-radioactive hosts the difference is not significant (Table 1). On this point, the most valid comparison for the dye + isotope series (93.34 ± 0.95) is with the dye injected control series (94.58 ± 0.87).

In contrast to hatchability results, a definite effect was demonstrated for larvae which had fed upon radioactive hosts as measured by their inability to complete this phase of development. Fewer larvae succeeded in pupating if fed upon caterpillars containing higher levels of radioactivity than if fed at lower levels. However, even though raw data have been converted into percentage of controls, variability between similar doses was appreciable as shown by the jagged plot of Figure 1. As suggested in the Discussion below, an aspect of the injection method may be responsible. In order to test whether the apparent trend is significant, regression coefficients and their significance were calculated for both the haploid and biparental series. Respectively, $t_{(41)} = 2.97$ and $t_{(12)} = 3.81$, both significant at the 1% level. Furthermore, if regression had been considered only for the high doses in the haploid experiment as has been done for biparentals, t would be greater. Pupation tends to approach control values below 600 μc per cc of host injected which results in about 4.8 μc ingested by the individual parasitic larvae.

Not infrequently naked pupae are seen in Habrobracon experiments. In the present case, records were kept of such occurrences during treatment at higher levels. No obvious relationship to dosage was revealed so these results have been pooled for summary:—

pupae from mated females, treated	7.0% naked
pupae from unmated females, treated	5.3% naked
pupae from mated females, control	5.2% naked
pupae from unmated females, control	0.2% naked

This suggests a slight effect of treatment. However the significant feature of naked pupation observed by us was that the ability to produce at least some "silk" was maintained. Instead of complete absence of "silk", the cocoon spun is incomplete so that the larva may leave it or the pupa may be easily dislodged from it.

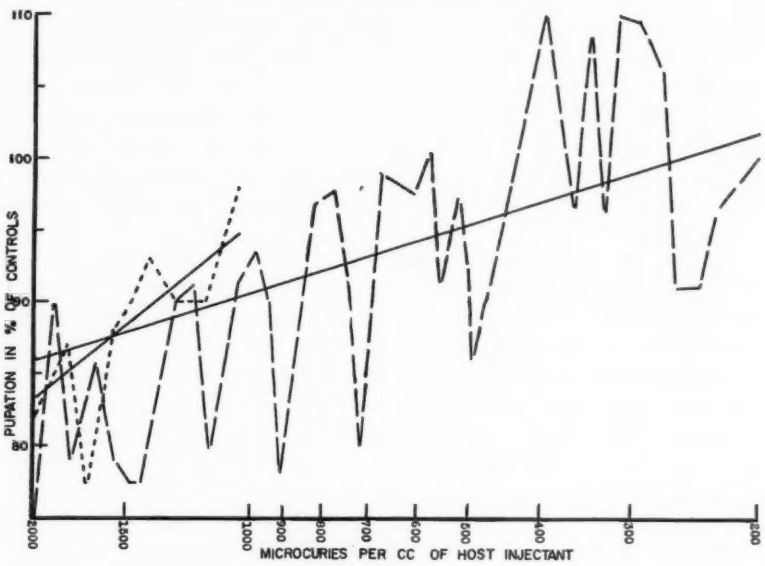

FIGURE 1

In per cent of control values, the pupation of haploid larvae (long dashes) and of larvae from mated females (short dashes) after rearing on radioactive hosts. Solid lines are the respective lines of regression. In order to estimate a level in microcuries per braconid larva, a given dose of host injectant in μc/cc may be multiplied by 0.008 cc per parasite. This conversion factor is based upon 0.04 cc introduced into host tissue which is ingested by 5 parasites. Points through which the broken lines are drawn are based upon the samples designated in level and size in Table 1.

The competence of experimental larvae to metamorphose is compared with control values in Figure 2. This is based upon the proportion of imagos achieving a free standing position. An adequately clear representation of results was obtained here when the points from the raw data were connected to form lines. In comparison with pupation results, metamorphosis is found relatively sensitive to internal radiation because here control values were approached only at levels below 300 μc per cc of injectant or 2.4 μc per parasitic larva. Furthermore, as shown by Figure 2, metamorphosis at 2000 μc/cc was only 25% of control values in contrast to pupation at 75% of controls (Figure 1). At other dosages similar contrasts are apparent.

Delay in the emergence from cocoons, usually termed developmental delay, became apparent in experiments above the 800 μc per cc injectant level. A summary in per cent of the total emerged reveals the following:—

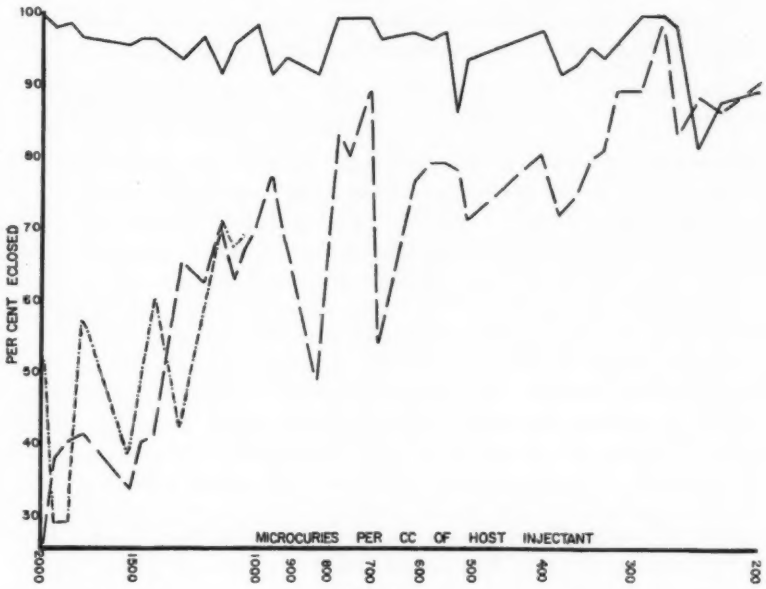

FIGURE 2

The eclosion of Habrobracon after ingestion of radiophosphorus. Average values for impaternate haploids are represented by the broken line of greatest length, and for biparentals by the dot-dash line. For comparison, control averages are given by the solid line. Again an estimation of the treatment level on an individual parasite basis may be obtained by multiplying host injectant values in μc/cc by the conversion factor of 0.008 cc. The animals of figure 2 are the same ones of figure 1 less those which have died.

Day	Ninth	Tenth	Eleventh	Twelfth	Thirteenth
Controls	76.95%	21.49%	1.56%		
Experimentals	43.43%	43.18%	10.94%	2.12%	0.30%

Upon inspection of those haploid males which failed to enclose, at doses above 800 μc per cc of injectant, 89.4% were found to be pigmented with unexpanded or incompletely expanded wings. Only 5.5% of the unemerged wasps were completely imaginal; a still smaller proportion, 4.5%, were prepupae; and only a very few, 0.5% were unpigmented pupae. The last category may be discounted because the unemerged pupae of controls were in a similar stage and proportionately numerous.

In experiments yielding biparental offspring the following unemerged types were found upon dissection of cocoons:—

Experimentals .. 116 males, 43 female, 135 unclassifiable prepupae
Controls ..... 32 males, 26 female, 22 unclassifiable prepupae

In addition the experimental cocoons yielded 3 gynandromorphs. If experimental results are compared with the proportions expected from control results, a high χ^2 of 78.12 ($P. < 0.01$) is obtained due to the appreciable deviation in the female and unclassifiable categories. This is taken as indication that the populations are not the same. In the adults which emerged, the sex ratio among controls, 52% male to 48% females, is not significantly different from the sex ratio in those controls which developed externally recognizable sex characteristics but failed to emerge. The χ^2 is 0.2338 with 1 d.f. and P greater than 0.50. In contrast to control results the sex ratio of adults emerged after treatment is found to be 39% males and 61% females, which is almost a complete reverse from the ratio demonstratable among dead pupae;—116 male to 43 female is 73% to 27% female. Thus relatively fewer males attain adulthood than do females under the conditions of the experiment and females are shown to be more resistant to radiation from an internal source.

At doses above 800 μc per cc of host injectant, abnormal morphology became apparent in a fraction of the emerged adults. Figure 3 gives an indication of this feature and shows the general relationship to level of radioactivity used in injections. We believe this to be the first depiction for Habrobracon of the quantitative relationship of induced developmental abnormalities to radiation level. Of a total of 120 abnormal wasps only 3 showed antennal abnormalities. Wing abnormalities accounted for the rest of the aberrations. Of the 117 with abnormal wings, 82 wasps showed unusual carriage of the wings. A summary of these types is classified as follows:—

27-32.9%	unilateral, primary and secondary wings extended.
26-31.7%	unilateral, primary wing extended.
18-21.9%	unilateral, secondary wing extended.
7-8.5%	three wings extended.
3-3.7%	all four wings extended.
1-1.2%	bilateral primary wings extended.
Total 82-100.0%	

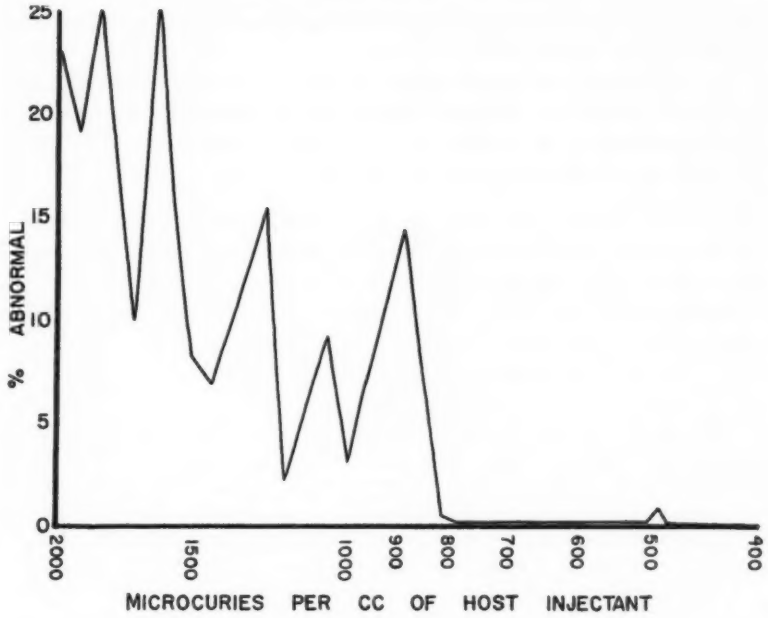

FIGURE 3

The morphologically atypical adults produced by rearing male Habrobracon larvae on radioactive hosts. An estimation of the treatment level on an individual basis may again be obtained by multiplying host injectant values by the conversion factor of 0.008 cc.

Hence unilateral unretracted wings was found to be the most common type of abnormality, the remaining 35 live adults with abnormal wings were comprised of 28 with wrinkled wings, 3 cases of completely unexpanded wings, 2 cases of non-expansion distal to the stigma, 1 with fluid filled wing sac and 1 with notched wings. That all 120 abnormalities were developmental effects upon somatic tissues and non-hereditable were demonstrated by crossing the affected males to wild type, isolating virgin daughters, and observing their haploid offspring. No transmission to future generations was obtained.

In contrast to the frequent occurrence of abnormal males, only 3 abnormal females were seen and these were at the highest dose. One showed abnormal carriage of a primary wing and 2 showed wrinkled wings. The rare occurrence of structural abnormality in the

female appears to be another indication of the relative radioresistance of this diploid type.

An observation on treated adults which is difficult to express quantitatively is that of unsteady stance and locomotion. However, as shown in Table 2, no striking effect was demonstrated on the life span of adult survivors of rearing on radioactive hosts.

TABLE 2

Life span in days at 30°C for male Habrobracon under starvation conditions as adults.

μc/cc of injectant	Host Larval Contents			
	P^{32} and dye	P^{32} and Ringers	Dye Alone	Uninjected
400-775	13.40 ± 0.06		13.21 ± 0.07	13.71 ± 0.06
816-2000	14.14 ± 0.23	14.28 ± 0.18		13.82 ± 0.12

In females derived from treated fathers, no evidence of an induced hereditary influence upon egg production was obtained. With controls producing an average of 12.02 eggs per day over the first 10 days of adulthood, females derived from

312 μc/cc of injectant gave 9.12 eggs per day.
400 μc/cc of injectant gave 18.64 eggs per day.
600 μc/cc of injectant gave 13.24 eggs per day.
800 μc/cc of injectant gave 12.55 eggs per day.
2000 μc/cc of injectant gave 9.76 eggs per day.

At the same time, the indication of induced embryo lethality was very slight. With control hatchability at 98.5%, the hatchability in the above eggs was 98.2%, 92.0%, 93.7%, 97.9%, and 98.0% respectively, corresponding to the order of doses given above. Pertinent here is the thought that a selection effect may be imposed through the considerable pupal lethality at higher levels of treatment. Damaged nuclear mechanisms may be mostly eliminated by their inclusion in organisms which do not survive metamorphosis.

Discussion

One feature of the injection technique which needs further attention before critical quantitative work can be performed is the leakage of injectant from the injection site. Although aware of it we have not been successful in devising methods for measuring it or halting it.

For one thing, if the diameter of the injection needle is too small, difficulty in delivering the injectant becomes great. To leakage we ascribe the variability in results reflected by the zig-zag lines on the figures. Two injections closely related in level of radioactivity of the injectant, conceivably may produce quite different results if the diameter of one of the needles or the mode of insertion into the body wall results in greater leakage. Nevertheless, the injection method surmounts a previous limitation in the scope of braconid experiments. Heretofore the intact paralyzed host has been considered to be the only acceptable source of food for braconid larvae. Now that a feasible method of introducing desired agents into the physiological interior of the host has been demonstrated and the product shown agreeable to feeding larvae, experiments parallel to those with other forms where agents are added to the rearing media may be achieved. Drosophila experiments command attention for comparison with developmental studies as the present, while in the future, when mutagenesis may be under consideration, results with microorganisms should prove useful.

Incidental observations concerning the effects on development of radioactive media appear in papers dealing primarily with other aspects (Blumel, 1950, for example). No attempt will be made here to survey the various items of this type which do little more than mention lethality or morphological aberrations. Instead, detailed reference will be made only to a pertinent report concerned primarily with developmental aspects:—the Arnason, Irwin and Spinks (1949) determination of tolerance limits by placing eggs of *Drosophila melanogaster* on media containing concentrations of P^{32} in graded series. As in the present results, food containing radiophosphorus resulted in lethality at developmental "crises" rather than immediately. Death occurred either toward the end of larval life or during the pupal stages. We believe that this type of result is traceable to effects on the cell nucleus since successful metamorphosis requires an extraordinary amount of mitotic activity and the functioning of endomitotic tissues.

Along these lines it should be noted that less radiation was required to halt development after pupation than to influence pupation in both the present study and that by Arnason *et al.* Measured by biological effectiveness, the top treatment levels employed by the Arnason group were somewhat greater than ours since they exceeded

levels which prohibit emergence of adult flies. In view of injection leakage, further quantitative comparisons with this Drosophila work do not seem in order at this time. However, a rough analogy is found in the decimal point of dosages sufficient to reduce emergence of adults when the Habrobracon and Drosophila results are calculated in common units.

If attention is shifted to results with rays from external sources, a considerable amount of work on developmental aspects in Habrobracon is available. This included two studies employing P^{32}. One is available only as an abstract of a preliminary investigation (Martin, Dent, and Joseph, 1948). The second (Dent and Amy, 1950) demonstrates that death after treatment usually occurs during pupation with an appreciable degree of inverse correlation between the extent of imaginal differentiation and the radiation exposure. Also noted was a reduction in the rate of development. Thus there are some points of similarity to present results, however, on other points there is divergence. Whereas Dent and Amy found the failure to form a cocoon correlated with treatment, the phenomenon did not occur in our experiments. Care was taken not to disturb spinning larvae and although we observed some naked pupae, such organisms had not lost the spinning ability. Rather, as termed by Hase (1922), a small percentage of both experimentals and controls wandered while spinning to produce a "street" of cocoon. An additional point of divergence is that while we find morphological abnormalities correlated with level of treatment, the Dent and Amy results show no significant increase of this feature with treatment.

If viewed from the standpoint of developmental cytology, the apparent inconsistencies are found to fit nicely into a logical pattern. In the Dent and Amy experiments, exposure occurred only for the embryo and the very young larvae. It is exactly during this time that mitotic and endomitotic activity of the spinning gland is at a height. On the basis that nucleinated, relatively contracted chromosomes confer radiation-vulnerability to cells in endomitosis as well as to those in mitosis (Grosch and Sullivan, 1954) the exposure was being made just at the period of sensitivity of spinning gland tissue. Nuclear activity in appendage buds of the type which produces vulnerability does not become appreciable until much later; that is in the late larvae and during the pupal periods. In fact, with exposure to external rays extended to include the entire developmental period,

a considerable number of developmental abnormalities were obtained. In discussion, Dent and Amy (1950) indicate such results to have been a feature of the Martin, Dent and Joseph (1946) experiments.

Due to the structure of the digestive tract (Grosch, 1949), material ingested by Habrobracon larvae cannot be egested until an advanced stage of imaginal morphogenesis has been achieved. Even then the meconium continues to be associated with the organism until eclosion. Thus by necessity the irradiation of the present experiment continued throughout the entire developmental period. Once ingested, only the half life of the isotope determines the level of treatment at any given time. Furthermore, the radiation dose within the organism continues to accumulate as each increment per unit time is added to that which has occurred previously. Fortunately for the spinning glands, they have long attained a state of high polyploidy before the accumulated dose can reach significant levels. However, while polysomaty is achieved in specialized larval tissues, the cells of the adult appendages continue with their basic chromosome set, haploid or diploid depending upon the sex type. Thus when adult anlagen become mitotically active the structures are relatively vulnerable. In the impaternate male, haploidy explains the greater sensitivity of its appendages. Further, lethality in the male may be traceable to the haploidy of significant tissues at critical periods in metamorphosis. It should be remembered that a record such as eclosion summarizes overall sensitivity by indicating those organisms which in spite of ever-increasing, accumulated radiation dose have passed all crises up to the time the record is taken.

Experiments with X-rays feature relatively brief exposure at a high intensity in comparison with the chronic type of exposure considered above. However present results are consistent with three general principles:—demonstrated in the work of A. M. Clark and associates on pupated forms (Clark and Kelly, 1950; Clark and Mitchell, 1951) and in the study by Whiting and Bostian (1931) of irradiated larvae. (1) Induced wing abnormalities become common when an appreciable amount of radiation is employed. (2) Delayed development is typical for irradiated organisms. (3) Haploids are more sensitive than diploids throughout larval and subsequent stages.

Summary

Radiophosphorus at 48 dilutions was injected by capillary needle into paralyzed Ephestia larvae. Habrobracon eggs were then transferred to these hosts as well as to controls, both injected and non-injected. At the levels investigated:—

(1) No significant differences in hatchability were obtained between eggs hatching on radioactive hosts and those on non-radioactive controls.

(2) A slight but definite effect is seen upon the percentage pupated particularly above 600 μc/cc of injectant. In addition to a lethal effect, naked pupae were observed. However such organisms had not lost the ability to produce silk but rather showed altered spinning behavior.

(3) The most obvious lethal effect was found in metamorphosis. Considerable decreases occurred in the numbers completing metamorphosis above 300 μc/cc of injectant.

(4) Developmental delay along with developmental abnormalities of the appendages became apparent above 800 μc/cc of injectant. Wing abnormalities were the most common.

(5) Females, the diploid type, were found to be more radioresistant than the haploid males, both in their ability to complete metamorphosis and also to do so without suffering structural aberration.

(6) Life span of animals achieving adulthood has not been shown to be affected.

(7) Also negative was an attempt to demonstrate a hereditary influence on egg production.

(8) A small amount of embryo lethality was demonstrated to be induced in the genetic mechanism. However measurement of this feature is complicated by the possible selection effect of pupal mortality.

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Growth, 1954, **18**, 207-214.

THE DEVELOPMENT OF A BREED OF MINIATURE SWINE —A PRELIMINARY REPORT[1]

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There is need in medical research for new laboratory animals which anatomically and physiologically resemble man. Such animals to be most useful must reproduce prolifically and be easily raised; they must also be easily maintained and handled under laboratory conditions. Except in the matter of size, the pig meets these requirements remarkably well. Therefore, in late 1949, a program was initiated at the Hormel Institute to develop a physiologically and anatomically normal breed of pigs which, due to their genetic constitution, would be unable to achieve an adult size of more than about 1/8 to 1/10 that of ordinary sized swine.

The specific objectives of the project are to produce (1) a prolific and easily handled laboratory animal which anatomically and physiologically closely resembles human beings, (2) a laboratory animal that might be susceptible to disease agents for which no satisfactory laboratory host is presently available, (3) a laboratory animal that will be useful in a wide variety of laboratory research, and (4) fundamental information about the genetics of swine and swine breeding.

METHOD OF PROCEDURE

This project is envisioned as a further test of the effectiveness of the flexible systems of inbreeding and selection used in producing the Minnesota No. 1 and Minnesota No. 2 breeds of ordinary-sized swine

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from crossbred foundations, since the basic structure—but not the content—of the genetic foundation of the miniature swine and the methods of inbreeding and selection that are being used to bring about desired changes in the genetic structure and content of the ensuing generations are the same as those pertaining to the Minnesota No. 1 and No. 2 breeds.

Three wild stocks have been crossed to provide a pool of widely divergent heredity as a foundation from which to develop a breed of miniature swine. Females of the so-called "Guinea Hog" stock that runs wild in Alabama were crossed with a boar of stock that runs wild on Santa Catalina Island. The former were black, short-legged, and very fat; the largest of these weighed more than 500 pounds at an advanced age. In conformation, the boar of Santa Catalina Island stock was wide in the shoulders and narrow in the rear quarters. At apparent maturity he weighed slightly under 400 pounds. The third stock used was the "Piney Woods" hog from Louisiana. Body conformation, growth rate, and color patterns indicate that this stock is the most genetically variable of the three wild stocks used. Average one year old weights of females of this stock raised at the Hormel Institute were about 240 pounds. Four Guinea Hog females, one Guinea Hog boar, and one Piney Woods boar died before use. The entire present herd traces back to six Guinea Hog females, one Catalina Island boar, and three females and one boar of the Piney Woods stock.

The plan of matings is as follows: in both two-breed and three-breed foundations, matings have been so made as to obtain a crossbred population carrying approximately equal percentages of heredity from each of the foundation stocks. Following the crossing necessary to obtain such a genetic structure, inbreeding is proceeding on a flexible basis whereby the best females are mated to the best males regardless of relationship rather than mating the best to the best within a specified pattern of relationship.

The major factors considered in selection are fertility (number born alive), survival, weight for age, and body conformation. Failure of sufficient attainment in any one of these traits results in culling. Early sexual maturity, and color and texture of skin are important within the above framework.

Management practices are essentially the same as those used in handling ordinary sized swine. Full feeding, self-fed, is practiced.

Performance testing to 154 days of age is practiced under as nearly uniform conditions as is practically possible.

A continuous system of farrowing is used with litters being farrowed each month. Some pigs of all generations are being produced at the same time as one of the consequences of practical factors affecting the operation of the program; this serves incidentally to stabilize the effects of differential environments on litters of succeeding generations.

Results and Discussion

Under the effective use of a given system of mating and selection, the rapidity with which a given goal can be achieved depends upon the frequency of favorable heredity in the stocks used as a genetic foundation. It is reasonable to believe that the frequency of heredity for small size has been decreased in our commercial breeds of swine through the selection pressure that has been exerted during the periods of their formation and improvement. Therefore, wild stocks that have been subject for many years only to the forces of natural selection should possess a higher frequency of heredity favorable to the development of a breed of miniature swine than would commercial stocks. It is further to be expected that through crossing wild stocks of diverse origins a greater pool of divergent heredity for small size will be obtained than is available through the use of only a single wild stock.

Three wild stocks, designated here as A, B, and C have been crossed to provide a genetic foundation for the miniature breed. Stocks A and B were obtained in late 1949; stock C was obtained in mid 1951. Table 1 presents data on weight for age by various groups; and Table 2 gives data on fertility and livability by various breeding groups.

The basic problems involved in developing miniature swine are those of (1) separating heredity for small size from that for large size and (2) reassembling heredity for small size into new combinations which produce a new and genetically stable breed. To do this requires (a) that the system of breeding be devised so as to induce maximum segregation and recombination of heredity for small size, and (b) that the system of selection provide recognition and utilization of the individuals possessing desired heredity.

To accomplish requirement (a) above, matings have been kept

TABLE 1

WEIGHT FOR AGE BY BREEDING GROUPS AND GENERATIONS IN THE MINIATURE PIG PROGRAM

Breeding groups	Birth weight (lbs.)			Weaning weight (lbs.)			154-Day weight (lbs.)		
	$\bar{x}$	S	CV	$\bar{x}$	S	CV	$\bar{x}$	S	CV
F_1 AxB	2.00	.48	24	20.7	8.0	38	113.6	18.7	16
F_2 AxB	1.81	.35	19	13.8	4.3	31	75.6	19.9	26
F_3 AxB	1.75	.51	29	13.6	4.7	35	42.8	8.5	20
F_1 AxBxC	1.92	.42	22	15.7	5.8	37	67.1	23.2	35
F_2 AxBxC	1.71	.46	27	11.4	4.1	36	50.1	15.3	31
F_1 BxC	2.23	.33	15	21.9	5.7	25			

TABLE 2

FARROWING DATA ON GILTS AND SURVIVAL DATA ON PIGS BY BREEDING GROUPS

Breeding group	Dams[1]			Litters[2]	
	Number of litters	Age at farrow (months)	Number born alive	Number of litters	Per cent survival[3]
F_1 AxB	18	12.6	6.17	13	73
F_2 AxB	10	11.2	7.50	21	53
F_3 AxB	6	13.0	5.83	7	50
F_1 AxBxC	17	11.2	6.53	32	63
F_2 AxBxC	6	11.3	7.33	28	61

[1] Gilts only.
[2] Both sow and gilt litters.
[3] These are of the same generation as the dams in the first three columns; they are not the same generation as number born alive (column 3).

wide—but not of constant width—within the limits imposed by the policy of mating the best to the best. This is practiced in conformity with the views that (1) segregation and recombination occur continually within the limits of the available heterozygosity and (2) very close inbreeding reduces the remaining heterozygosity rapidly and inevitably thereby reduces the opportunity for the segregation and recombination upon which opportunity for progress depends. A flexible system of selection in which there is no set proportion of aged sows to gilts and no fixed proportionate value of any one trait to any other trait has been used in an attempt to meet requirement (b) above. The variation expressed indicates an effective level of segregation and recombination of heredity; the decrease in weight at all ages with

succeeding generations (Table 1) and the farrowing performance (Table 2) indicate that selection thus far is resulting in recognition and utilization of individuals possessing desired heredity.

The data in Table 1 reveal (1) a marked decrease in weight for age with each succeeding generation arising from the crossbred foundation of AxB and AxBxC; and (2) that the three-way cross is superior to either of the two-way crosses as a genetic foundation for developing a breed of miniature sized swine; that is, that each of the three stocks used for crossing is contributing heredity favorable to attaining small size. The standard deviations and coefficients of variation present a picture in conformity with genetic expectations in view of (1) the effect of a crossbred dam on early performance of the litter and (2) the deliberate attempt to follow a mating plan which will induce as much segregation as possible. It is further apparent that not only is the three-way cross superior to the two-way cross from the standpoint of size but also from the standpoint of variation in size which is so essential to progress in the early stages of the breeding program. In view of the above, all litters are now being farrowed from a three-way-cross background.

FIGURE 1

A miniature pig at 154 days of age, weighing 25 pounds. Note trimness and soundness, and appearance indicative of general good health. (Background 4 inches/square).

Figures 2 and 3 provide striking illustrations of the ranges in weight at 154 days of age by generations; the marked shift in distribution of the populations on the weight curves is also evident. The results indicate that selection for small size has been effective in each selected generation. It is pertinent to point out in this regard that due to the need to (1) increase the number of females in the breeding herd, and (2) replace the foundation animals, selection of female breeding stocks on the basis of size has been less strenuous than would be possible under other conditions. Stringent selection of boars has been practiced.

Table 2 indicates that the number of pigs born alive per litter is at least holding its own and perhaps is increasing. This trait is affected significantly in ordinary sized swine by age at farrow; it seems reasonable to expect that the same relationship exists in the miniature pig. The age at farrow has been decreased in later generations.

Livability exhibits no definite change and is especially difficult

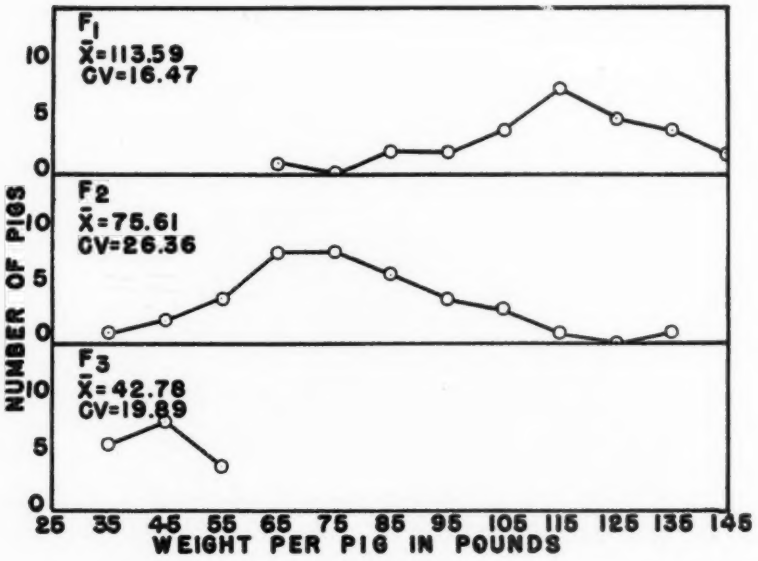

FIGURE 2

AxB Foundation—Range and distribution of weights at 154 days of age. Compare with Figure 3.

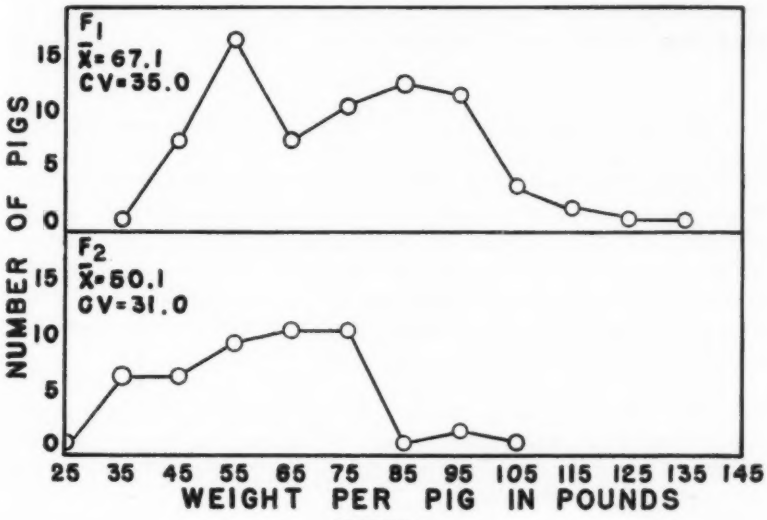

FIGURE 3

AxBxC Foundation—Range and distribution of weights at 154 days of age. Compare with Figure 2.

to evaluate under conditions at the Hormel Institute. Analysis of mortality data shows that the smaller pigs within each litter, on the average, have a lower livability than the larger ones. Competition and inadequate temperature control alone are sufficient to account for this situation. It is the observation of the herdsman and others who observe these very small pigs at birth, even those weighing less than a pound, that they are unusually active and "vigorous." It appears that per unit of size inherent vigor has not declined; it appears rather that an adjustment of intra- and extra-litter environment to the requirements of the smallest pigs is needed. During the period January-May, 1954, inclusive, 127 pigs farrowed by hysterectomy and reared artificially in isolation as described by Young et al. (1) have shown a livability of 87%.

Body form, along with size, has shown marked change toward the ideal which most medical research workers have indicated is desirable from their standpoint. Surplus pigs and those not desirable as breeding stocks are being used in a number of laboratories for various medical studies. Users report favorably upon adaptability and man-

agerial requirements as well as physiologic and anatomic suitability of these pigs for laboratory purposes.

Summary

A program to develop a breed of anatomically and physiologically normal miniature swine for use in medical and other laboratory research work was initiated in 1949 at the Hormel Institute. Three wild stocks have been used to provide crossbred foundations that can be expected to contain a relatively high frequency of divergent heredity for small size. Flexible systems of inbreeding and selection based on performance for fertility, survival, weight for age, and body conformation are being used to bring about segregation and recombination and eventual fixation of desirable combinations of genes.

In the two breed cross foundation, average 154-day weights have declined from 114 pounds in the first generation to 43 pounds in the third generation. In the three breed cross foundation, average weights to date at 154 days are 67 pounds and 50 pounds for first and second generation pigs respectively.

General improvement has been noted in number born alive and in age at first farrow. No definite trend is apparent in livability data. Wide segregation is occurring for size and body conformation. Surplus pigs are being used for various medical purposes in a number of laboratories.

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HISTOLOGY OF THE ANTERIOR PITUITARY GLAND OF THE FROG DURING NORMAL DEVELOPMENT[1]

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INTRODUCTION

The existence of three traditional cell types—acidiophils, basophils, and chromophobes—in the anterior pituitary of vertebrates has been known for a number of years. However, as far back as 1932, Rucker and Wolfe demonstrated at least four cell types in the anterior pituitary of the domestic sow, albino rat, and the rabbit. Dawson (1938), Copeland (1943), Hartmann, Fain and Wolfe (1946), and Halmi (1950) have substantiated the presence of at least four cell types in the anterior pituitary of various species of vertebrates.

Recently, Romeis (1940) published the results of an extensive study of the anterior pituitary of several species of vertebrates. He reported that after staining with the kresofuchsin-azan technique of Berlinger and Burgdorff (1935), at least five cell types could be detected in the anterior pituitary of the rat.

Gomori (1950) showed that aldehyde-fuchsin will selectively differentiate between the beta and delta cells in the anterior pituitary of the rat and this has been confirmed by Halmi (1950), and Purves and Griesback (1951). Goldberg and Chaikoff (1952) using the aldehyde-fuchsin stain and a modification of Martin's hematoxylin-trichrome technique on the anterior pituitary of female dogs, found in addition to the five cell types described by Romeis (1940), a sixth cell type, designated the zeta cell.

Wilson (1952) emphasized the difficulty encountered in evaluating cytological studies of the pituitary gland, including differentiation of cell types present, because of the confusion resulting from the use of a variety of specialized staining techniques.

[1] An abridgement of a dissertation submitted in partial fulfillment of the requirements for the Master of Science degree in Zoology.

It is the object of the present investigation to attempt to differentiate the cell types present in the anterior pituitary gland of the frog during normal development.

Materials and Methods

Adult female and male frogs of the species *Rana pipiens* were obtained from the Lemberger Company of Oshkosh, Wisconsin. The pituitary glands were removed from seven female frogs and by means of a syringe and No. 18 needle the glands were injected into the lower quadrant of the abdomen of two healthy female frogs. The gonads of five male frogs were removed and a sperm suspension was made in a large finger bowl. After 72 hours, the eggs were stripped directly into the bowl containing the sperm suspension.

The fertilized eggs developed through metamorphosis to the adult stage. All dead tadpoles were removed. Food for the tadpoles consisted of strained liver soup. The water containing them was changed daily and the containers were cleaned every two days. At selected ages the tadpoles were removed to a fixative of nine parts saturated aqueous mercuric chloride and one part formalin. The stages included the following: (a) tail fin circulation; (b) completion of the operculum; (c) appearance of the hind limb bud; (d) appearance of digits in the hind limb; (e) immediately after metamorphosis; (f) the adult.

After metamorphosis the heads of several young frogs were removed and fixed in the same manner as the tadpoles. The pituitary glands were removed in the case of the adult frogs and fixation was the same.

Serial sections were cut at 5 μ, and stained with a combination of aldehyde-fuchsin and a modification of Martin's (1933) hematoxylin-trichrome as described by Goldberg and Chaikoff (1952).

Results

A classification consisting of lettered labels and subnumbers was used to denote the various cell types found in the anterior pituitary gland of the frog, based on cytological characteristics of the cell components. Cell types of the A, B, and C series represent the different kinds of acidophils, basophils, and chromophobes respectively.

Description of each cell type:

Cell Type A—

These small cells were variable in form, being round, oval or irregular, with the majority tending to be round or oval. The cytoplasm was fuchsinophilic and the fine cytoplasmic granules were purple.

The round to oval fuchsinophilic nuclei contained 1-3 orange nucleoli and dark chromatin granules that were most numerous toward the periphery of the nuclei. The nuclear membrane showed signs of indentation in a number of these cells.

Cell Type A_1—

These were extremely large cells that contained very large round or oval fuchsinophilic nuclei. The cytoplasm was an orange purple and contained purple and orange granules that were evenly distributed throughout the cytoplasm. No nucleoli were observed in the nucleus. In a number of sections, these cells tended to be arranged in rosettes and the cell boundaries were more or less distinct.

Cell Type A_2—

These cells were variable in shape, but most of them were polygonal. The coarse dark purple chromatin granules of the fuchsinophilic nuclei were most numerous toward the center. There were from 1-2 fuchsinophilic nucleoli present in this cell type. The cytoplasm was light purple and contained fine light purple granules. In some instances the cytoplasm was scanty in this cell type.

Cell Type A_3—

These cells were similar in size to other members of series A, but were readily distinguished from them by the presence of a bright blue cytoplasm and bright red cytoplasmic granules.

Cell Type B—

These cells were round or oval and were arranged in rosette like patterns. They were smaller than members of series A. The dark purple nuclei contained extremely large blue purple chromatin granules.

The cytoplasm stained orange purple and dark blue cytoplasmic granules were present. Distinct cell boundaries were not detected in this type.

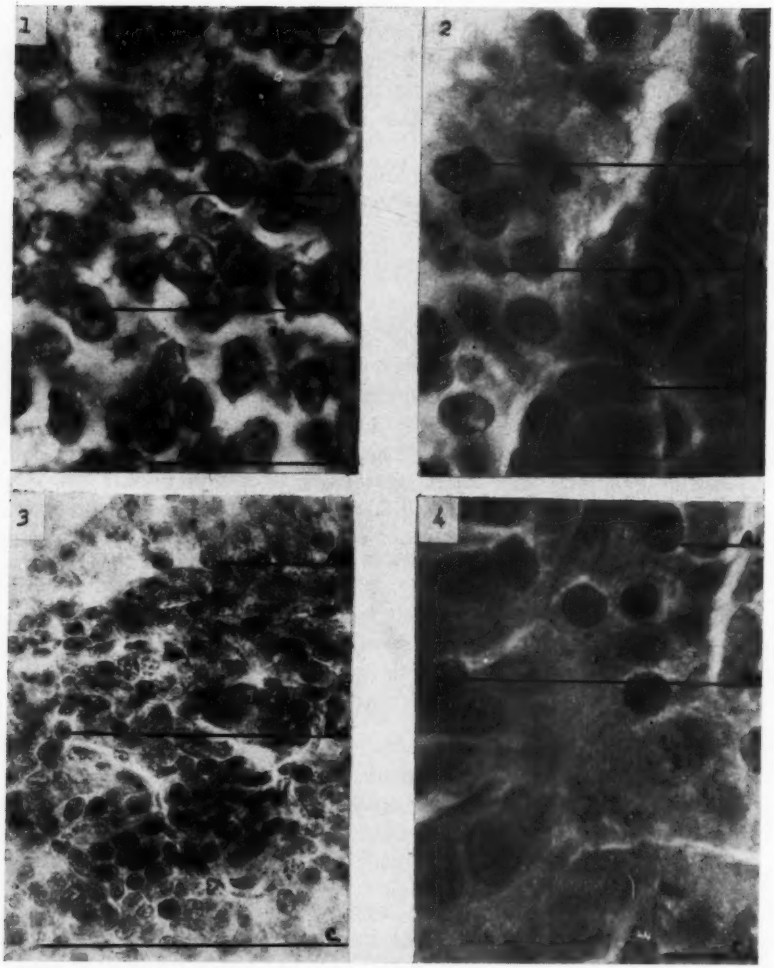
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PLATE 1
Explanation of Figures

FIGURE 1

Photomicrograph of the normal anterior pituitary gland in *Rana pipiens* at the completion of the operculum. Cell types A, B, B_1, and C_1 are present in this stage. X 1350.

FIGURE 2

Photomicrograph of the normal anterior pituitary gland in *Rana pipiens* at the hind limb bud stage. Cell types A_1, A_2, and C_2 are present in this stage. X 1350.

FIGURE 3

Photomicrograph of the normal anterior pituitary gland in the adult *Rana pipiens* immediately after metamorphosis. Cell types A_2, B_2, and C are present in this stage. X 430.

FIGURE 4

Photomicrograph of the normal anterior pituitary gland in the adult *Rana pipiens*. Cell types A_3, B_3, and C_3 are present in this stage. X 1350.

LEGEND

Fixation in Dawson's fluid.

Serial sections at 5μ.

Stained with a combination of aldehyde-fuchsin and a modification of Martin's (1933) hematoxylin trichrome.

A, A_1, A_2, A_3—types of acidophils.

B, B_1, B_2, B_3—types of basophils.

C, C_1, C_2, C_3—types of chromophobes.

Cell Type B_1—

These cells were smaller in size than those of type B; a single purple orange nucleolus was present in the dark purple nucleus. The cytoplasm stained a dark red to orange fuchsin and contained dark purple granules. The chromatin granules were similar in color to those present in cell type B.

Cell Type B_2—

These cells were similar in most characteristics to cell types B and B_1. However, the cytoplasmic granules in cell type B_2 were quite different from those of the previous members of series B, in that they were extremely bright blue in color.

Cell Type B_3—

These cells were easily distinguished from cell types B, B_1 and B_2 by the presence of a bright blue cytoplasm and dark fuchsin cytoplasmic granules. Other characteristics of this cell type were similar to the previous members of series B.

Cell Type C—

These cells were smaller than other members of series A or B. However, they were more elongated than cell type A. The nuclei did not stain very intensely. The nuclei, however, contained 1-3 orange nucleoli and the fine chromatin granules were light purple. The cytoplasm of these cells was very scanty, although it contained very fine light purple granules.

Cell boundaries were indistinct.

Cell Type C_1—

These cells contained very large nuclei that were irregular in shape and stained pale orange. The chromatin granules were purple. The cytoplasm was very scanty and no cytoplasmic granules were detected.

No cell boundaries were observed.

Cell Type C_2—

These cells were small and similar in shape to cell type C. The nuclei did not stain very intensely, but contained a few dark chromatin granules. The cytoplasm, however, stained a blue purple and very fine dark granules were observed.

Cell boundaries were more or less distinct.

Cell Type C_3—

This cell type was similar in shape to cell type C, and C_2. The nuclei did not stain very intensely, but fine dark blue chromatin granules were detected. The cytoplasm was pale orange fuchsin and contained fine light purple granules.

TABLE 1
SUMMARY OF DEVELOPMENTAL STAGES AND PER CENT OF CELL TYPES PRESENT

Developmental Stages	Percentage of Cell Types Present											
	A	A_1	A_2	A_3	B	B_1	B_2	B_3	C	C_1	C_2	C_3
Tail Fin Circulation	95%	—	—	—	—	—	—	—	5%	—	—	—
Completion of Operculum	60%	—	—	—	30%	5%	—	—	—	5%	—	—
Hind Limb Bud	—	25%	55%	—	5%	—	—	—	—	—	15%	—
Appearance of Digits in Hind Limb	40%	—	—	—	5%	50%	—	—	5%	—	—	—
Immediately after Metamorphosis	25%	10%	5%	—	—	—	55%	—	5%	—	—	—
Adult	5%	—	30%	5%	—	—	20%	25%	10%	—	—	5%

DISCUSSION

Wilson (1952) in a review article suggested, on the basis of some evidence, that the azan technique may be especially capricious when applied to early developmental stages of the gland. The greatest problem here lies in the variation in basophilic staining.

As has been noted, here the aldehyde-fuchsin stain combined with Martin's hematoxylin-trichrome technique (1933) has been used successfully to distinguish a number of cell types in the anterior pituitary of the frog at various stages. However, since a variety of developmental stages were used it was impossible to correlate, in all cases, the cell types found here with Romeis' (1940) classification, since most of the previous studies dealt with only the adult pituitary of various amphibians and mammals. However, the presence of two distinct staining areas mentioned previously and noted as early as the completion of the operculum stage, is in agreement with the conclusions of Rahn and Painter (1941).

The A, A_1, and A_2 cells designated here, corresponded to some degree with the alpha cells of Romeis' classification. There were, however, differences, since the A, A_1, and A_2 cells contained from 1-3 orange nucleoli in some cases instead of a single fuchsinophilic nu-

cleolus. The presence of from purple, orange-purple, blue and bright red cytoplasmic granules also deviated from the characteristic orange granules of Romeis' classification. Dawson (1939), working with the anterior lobe in the rabbit, monkey, and cat, Rahn and Painter (1941) with the chicken and duck, Copeland (1943) with the red salamander, and Hartmann, Fain and Wolfe (1946) with the dog demonstrated two classes of acidophils in these animals. D'Angelo (1940), using Dawson's staining technique, revealed that in the hind limb bud stage of several species of Amura, the anterior pituitary was predominantly acidophilic. The findings in the present work for the same stage in *Rana pipiens* were in accordance with the observations of D'Angelo. Wilson (1952) employed a modified protargol stain and demonstrated the presence of two different types of acidophils in the adult pituitary gland of the chick. It may be possible that the subgroups of cell type A, appearing after the tail fin circulation stage, were all derived from the same immature acidophil that was noted in the anterior lobe at the tail fin circulation stage.

The cell type C and its subgroups may be related to the gamma cell of Romeis' classification. These cells according to Romeis were small, not well demarcated, relatively chromatin-poor, and seldom found to contain a prominent nucleolus. Members of cell type C exhibited some of the characteristics of the gamma cell with the exception that in a few cases, the cytoplasm was either a pale blue orange, or pale orange fuchsin, and the nuclei contained 1-3 small fuchsinophilic or orange nucleoli.

There has been a great deal of confusion in the attempts to differentiate clearly between the beta and delta cells of Romeis. Goldberg and Chaikoff (1950) and Gomori (1950) were unable to demonstrate clearly the beta and delta cells (both basophils) of the dog using the kresofuchsin-azan stain of Berlinger and Burgdorff (1935). However, Gomori (1950 b) and Goldberg and Chaikoff (1952) have shown that aldehyde-fuchsin will selectively differentiate the beta and delta cells in the anterior lobe of the rat and dog. Copeland (1943) and Halmi (1950) were able to differentiate between the two types of basophils in the red salamander and rat respectively. Wilson (1952), employed a modified Bodian protargol stain, and demonstrated the presence of three different types of basophils in the anterior lobe of the adult pituitary of the chick.

According to Romeis' classification, the beta cells contained 1-3

fuchsinophilic nucleoli and the cytoplasmic granules were concentrated at the periphery of the cell. In contrast, his delta cell contained a single fuchsinophilic nucleolus and the cytoplasmic granules were homogeneously distributed throughout the cytoplasm. In the present work, cell type B contained a single fuchsinophilic nucleolus, but the cytoplasmic granules were concentrated at the periphery of the cell. Cell types B, B_2, and B_3 contained 1-3 large fuchsinophilic nucleoli, but the cytoplasmic granules were scattered evenly throughout the cytoplasm. Here again is another example of one of the chief difficulties encountered in correlating staining characteristics with specific cell types in the anterior pituitary gland, especially when attempts at such correlation cross generic limits.

The A_3 cell type in the adult stage of the frog corresponded in some degree to the epsilon cell of Romeis. The presence of bright red cytoplasmic granules, indented nuclei, and coarse chromatin clumps gave this cell a character similar to that of his epsilon cell. However, the A_3 cell contained 1-3 orange nucleoli and a bright blue cytoplasm. No cell types were observed here that corresponded to the zeta cell found in the anterior pituitary of dogs, as demonstrated by Goldberg and Chaikoff.

It is difficult to state positively, that there is a definite cycle involved in the decrease and increase in per cent of related cell types noted in the different developmental stages. However, if one can reasonably assume on the basis of morphological features that the A cells, B cells and C cells represent three separate lineage lines, then a cycle of cell type populations can be worked out which may have some significance. This conclusion, however, is merely tentative since it is based on cell groupings which thus far do not have strong experimental support.

The highest per cent of cell type A series was noted in the early stages, but there was a decrease which occurred just before metamorphosis and which continued through the adult stage.

However, the highest level of members of cell type B appeared just before metamorphosis. There was a decrease in the per cent of series B in the adult stage. It is possible that the high level of members of cell type B just before metamorphosis in the tadpole, was correlated to the completion of the various changes that occurred during metamorphosis.

There was less variation in the per cent of cell type C series in the

various developmental stages. The lowest level was noted in most of the early stages and remained rather constant, but there was a slight increase in the adult stage.

The assignment of specific products to the various cell types of the pituitary has been and still is a point of controversy. Most workers agree that the alpha cells of Romeis' classification produce the growth hormone and the basophils (beta and delta) were considered by most investigators to be responsible for both TSH and FSH production. Halmi (1950), Purves and Griesbach (1951) do not agree as to which is secreted by the beta and which by the delta cells.

Lacour (1950) suggested that the epsilon cell produced the lactogenic hormone. The question as to which cell type in the anterior pituitary gland is responsible for ACTH secretion is quite unsettled. There is still a great deal of confusion involved in attempting to correlate the various cell types with hormone production in the frog and other lower animals. Therefore, there is need for further study on the anterior pituitary in order to determine clearly the relation between various cell types and specific hormone production, and other physiological functions.

Summary

1. The histology of the anterior pituitary gland of the frog in various developmental stages and in the adult stage was studied.
2. The total cell types varied from two in stage 1, to seven in stage 6.
3. An attempt was made to correlate the cell types here with those of Romeis' classification.
4. The stain used was a combination of Martin's hematoxylin-trichrome and aldehyde-fuchsin (1933).

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SOME EFFECTS OF X-IRRADIATION OF DIFFERENT EMBRYONIC STAGES OF THE TROUT (SALMO GAIRDNERII)

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INTRODUCTION

The following study on rainbow trout, *Salmo gairdnerii*, contains the results of a preliminary investigation into the effects of X-rays on six somewhat arbitrarily chosen developing stages of the embryo. It seemed important to seek out, not only the most radio-sensitive states and the resultant effects of various doses of X-ray, but also the degrees of sensitivity of the embryo as it developed through the critical hatching and feeding stages. Since hard ionizing radiations uniformly penetrate all parts of the organism, selective response of structures may be expected to indicate patterns of sensitivity intrinsic in the embryo. The experiments were planned to determine the period or periods in development of the embryo when radiation would be most damaging to the organism.

BACKGROUND

There is considerable literature dealing with the treatment of developing animals with injurious agents. A large portion of this literature deals with the effects of radiation on mammalian and amphibian embryos. Hard ionizing radiations with the power to penetrate the embryo, the accurate timing of both onset and duration of treatment and the possibility of quantitative comparisons of different doses has caused a number of investigators to use X-rays as a tool to increase our knowledge of the developing embryo. Early in these investigations it became apparent that there was an inverse relationship of increasing age and sensitivity to ionizing radiations (Butler, 1936). Later investigators (Rugh, 1954; Wilson, 1954; Russell and Russell,

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1954) studied the differential sensitivity of the developing embryos of amphibia, rats, and mice and discovered that certain "critical periods" existed during which the embryo was more sensitive to radiation than at any previous or later period of development. In some of the experiments exact correlation of radio-sensitivity with age of embryo was not complete, at least in some early stages of rat embryos (Wilson, 1954), and in the later stages of amphibians (Rush, 1954).

In reviewing the work on the effects of radiation on fishes we find very few concerned with the embryological phases (Solberg, 1938; Welander et al., 1948; Foster et al., 1949). The differential sensitivity of the embryological stages and the quantitative effects at stages earlier than the pre-hatching stage were not investigated. At present we have information regarding effects of X-rays on the gametes (Foster et al., 1949), on the eyed stages (Welander et al., 1948), on fingerlings (Bonham et al., 1948), and on the adults (Welander et al., 1950) of salmonoid fishes. It became apparent that other embryonic stages should be investigated in order to get a better understanding of the effects of X-rays on the more radio-sensitive parts of the life-history.

Experiments with teleost embryos using chemicals and various types of physical agents (temperature, light) have indicated certain critical stages (Rollefsen, 1930; Hata, 1927; Vernidub, 1939) or periods at which these agents are most injurious or effective. It is intended to compare these critical periods with those that are found in experiments with X-radiation.

Some of the prime factors which make the salmonoid embryo a very suitable animal for experimentation of the type outlined above are the extended length of time in the developmental period (about 50 days), the semi-transparent nature of the egg (enabling the researcher to identify any developmental period), and the comparatively large size of the egg (5 to 9 mm). Other factors are the number of eggs obtainable from the same female (3000 to 7000), the use of well-known standard hatchery techniques for raising the eggs and larvae, and the comparative abundance of basic knowledge available on the embryology of these fishes.

Materials and Methods

The rainbow trout eggs used in this experiment were the progeny of four females and eight males, two males being used to assure fertilization of the eggs of a single female. The fish were part of one

generation of a stock that had been selectively bred for many years at the University of Washington School of Fisheries.

Data relative to exposure to X-rays are listed in Table I and additional radiation factors are as follows. Two hundred kvp X-rays were delivered by a Coolidge tube with the intensimeter removed. Using 1.00 mm aluminum and 0.25 mm copper filters and a current of 20 milliamperes, the intensity obtained at 40 cm was approximately 133 r per minute as measured in air with a 250 r capacity Victoreen thimble chamber. Between 35 and 100 eggs with diameters averaging approximately 5 mm were irradiated in petri dishes in water which did not completely cover the eggs. The eggs were confined to the circular cross-section of the beam by a celluloid ring. A total of 4,011 eggs were used in this experiment.

The stages of development at which the six groups of egg lots were irradiated are illustrated by figures 1 to 6 inclusive. Samples of each group were examined and preserved at the time of irradiation to verify the average stage of development. Temperature units (Wallich, 1901) were sufficiently useful to estimate the time at which certain developmental stages occur in the salmonoid egg (see Figure 7). Complete development through hatching required about 532 t. u. for eggs from female No. 250, 545 t. u. for eggs from females No. 228 and 238, and 571 t. u. for eggs from female No. 226. The temperature of the water during the experiment averaged 57.7° F and ranged from 50.5° to 66.0° F.

The eggs were held in standard cement hatchery troughs with inside dimensions of 457 cm by 30 cm by 20 cm. In these troughs the eggs of each lot were placed in small (10 cm by 10 cm by 3 cm) plastic screen trays which were suspended about one inch above the bottom of the trough in standard hatching baskets. During exposure to radiation the eggs had to be handled with extreme care. Salmonoid eggs are very susceptible to physical damage, especially at certain stages (Vernidub, 1939; Trifonova, 1939; Hata, 1927). In order to expose the eggs to X-rays they were first carefully poured into petri dishes, the transfer taking place under water. The petri dish, filled with water to the top of the eggs, was then carried to the X-ray machine, exposed, and the eggs returned to the original basket. Except for irradiation the controls of each group were treated similarly. The length of time the eggs were in the petri dish and exposed to irradiation varied from 0.55 to 7.0 minutes and the time necessary to

TABLE 1

Dose, radiation intensity, temperature of the water, number of embryos, and stages of development during irradiation.

Female No. and date of spawning	Stage of development	Lot No.	1	2	3	4	5	6	7	8
226	One-cell	Dose	0r	25r	50r	100r	200r	400r	800r	1600r
(4/19/51)	(1.54 t. u.)*	r/m		136	136	136	390	390	390	390
		Temp. F		51.5	51.5	51.5	51.5	51.5	51.5	51.5
		Hours old		2	2	2	2	2	2	2
		No. eggs	266	86	80	82	92	78	85	94
238	Thirty-two	Dose	0r	25r	50r	100r	200r	400r	800r	1600r
(4/19/51)	cell	r/m		133	133	133	380	380	380	380
	(13.9 t. u.)*	Temp. F		50.5	50.5	50.5	50.5	50.5	50.5	50.5
		Hours old		18	18	18	18	18	18	18
		No. eggs	144	81	53	66	64	85	83	76
238	Early germ	Dose	0r	25r	50r	100r	200r	400r	800r	1600r
(4/19/51)	ring	r/m		138	138	138	138	138	400	400
	(97.8 t. u.)*	Temp. F		52.0	52.0	52.0	52.0	52.0	52.0	52.0
		Hours old		113	113	113	113	113	113	113
		No. eggs	165	70	87	85	81	86	87	77
250	Late germ	Dose	0r	40r	79r	158r	319r	634r	1285r	2570r
(4/25/51)	ring	r/m		132	132	132	132	390	390	390
	(124.7 t. u.)*	Temp. F		53.6	53.6	53.6	53.6	53.6	53.6	53.6
		Hours old		148	148	148	148	148	148	148
		No. eggs	155	79	87	80	77	76	80	81
250	Early eye	Dose	0r	38r	75r	150r	300r	600r	1200r	2400r
4/25/51	(332.1 t. u.)*	r/m		137	137	137	137	390	390	390
		Temp. F		56.0	56.0	56.0	56.0	56.0	56.0	56.0
		Hours old		360	360	360	360	360	360	360
		No. eggs	157	81	70	81	80	81	79	82
228	Late eye	Dose	0r	38r	75r	150r	300r	600r	1200r	2400r
(4/19/51)	(426.9 t. u.)*	r/m		135	135	135	135	390	390	390
		Temp. F		56.0	56.0	56.0	56.0	56.5	56.5	56.5
		Hours old		479	479	479	479	479	479	479
		No. eggs	79	35	38	35	35	37	35	38

* t. u.—Temperature unit, an increase of one degree above 32°F for a period of one day (Wallich, 1901).

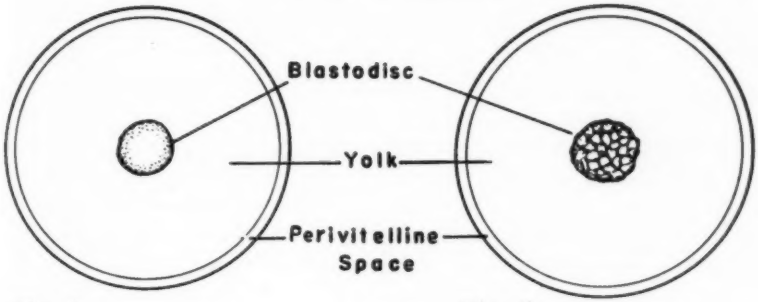

Fig. 1 Fig. 2

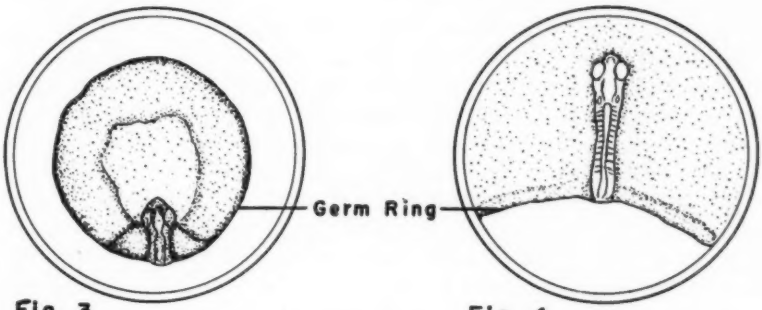

Fig. 3 Fig. 4

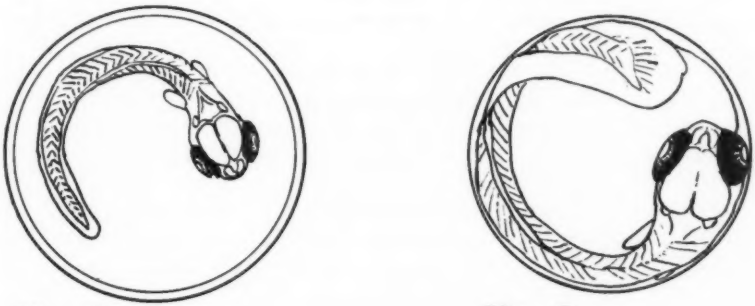

Fig. 5 Fig. 6

FIGURES 1 TO 6

Stages of development at which the six groups of eggs were irradiated. Figure 1 represents the one-celled or blastodisc stage, two hours after fertilization. Figure 2 represents the 32-cell stage, 18 hours after fertilization. Figure 3 represents the early germ ring stage, about 1/8th epiboly, 113 hours after fertilization. Figure 4, late germ ring stage, about 2/3rds epiboly, 148 hours after fertilization. Figure 5, early eye stage, 360 hours after fertilization. Figure 6, late eye stage or pre-hatching, 479 hours after fertilization.

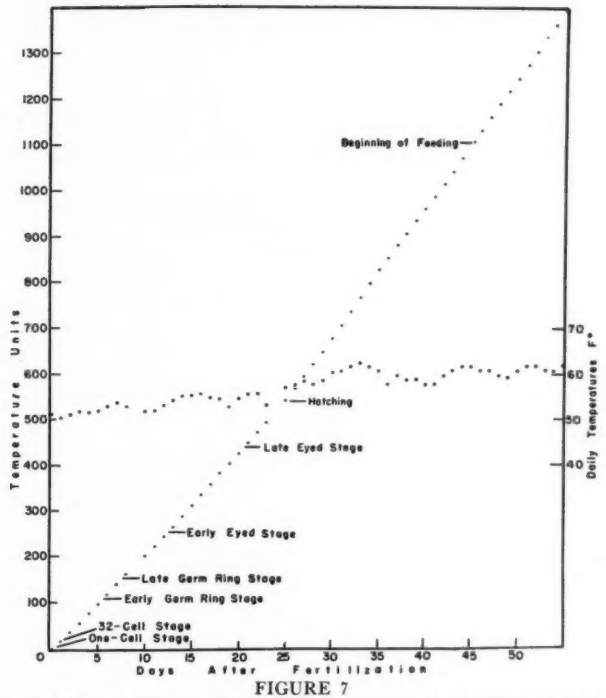

FIGURE 7

Relation of daily temperatures and temperature units to development of rainbow trout.

irradiate seven lots of a group of any one stage of development was about 30 minutes. In order to reduce temperature rise in the water in the petri dish during irradiation, the dish was placed in the X-ray beam in a large culture dish filled with water of the same temperature as the troughs.

After hatching each lot was removed to a 19 cm by 20 cm by 30 cm space in a hatchery trough divided into 24 such compartments by stainless steel screens. The larvae were kept in these compartments until they had used up the yolk and had begun to feed.

The eggs and larvae were examined daily and dead eggs were removed and preserved for study. At the end of 54 days (ca. 1400 t. u.) the experiment was terminated by weighing, measuring and preserving all surviving fish for future studies.

RESULTS

Mortalities

In this work death was judged by the change of the embryo or larvae from a semi-transparent appearance to an opaque or white color. In some cases death did not occur immediately in fish almost white in color, but the fish usually died within 24 hours. The yolk usually turns white or opaque in dead and dying eggs and larvae and can be used as an indication of death when the embryo is too small to be seen clearly. In embryos in which the heart is visible absence of heartbeat was sometimes used as a criterion of death.

The cumulative percentage mortalities were calculated from daily mortality data. This material is illustrated for the six irradiated stages. (Figure 8). It is obvious that the different stages show a differential sensitivity to radiation with a general decrease in sensitivity with age. Hatching and the beginning of feeding (after the yolk material is used) mark a definite and critical change in the life of the embryo and larvae and are used in this paper as reference points in summarizing the effects of irradiation (i.e., LD_{50} dose). Calculations were made of the median lethal dose after hatching and at the end of the experiment, using Karber's (1931) method, in the manner of Irwin and Cheeseman (1939), after adjusting for mortalities in the controls (Finney, 1944). These data are given in Table 2. The data tend to substantiate the results obtained by many other investigators (Butler, 1936) who found decrease of sensitivity with age. It is of especial interest to note that the doses which bring on mortalities after irradiating early cleavage stages are surprisingly low in the case of a cold blooded vertebrate and are comparable to doses which affect mammals irradiated during a similar period of development (Russell and Russell, 1954).

TABLE 2

Median effective dose (LD-50) calculated just after hatching and at the end of the experiment after the absorption of the yolk.

Stage irradiated	LD-50 at hatching	$\bar{S}x$	LD-50 at end of yolk stage	$\bar{S}x$
One-cell	78.3 r	± 4.4 r	57.8 r	± 3.8 r
Thirty-two cell	468. r	±19.4 r	313. r	±12.4 r
Early germ ring	524. r	±22.1 r	461. r	±15.9 r
Late germ ring	735. r	±24.7 r	454. r	±19.4 r
Early eye	——	——	415. r	±22.0 r
Late eye	——	——	904. r	±38.5 r

The distribution of the LD-50's in Table II indicate a differential sensitivity which does not decrease in direct relation with age of the embryo, and there is a considerable difference between LD-50's calculated at hatching and those at 55 days. If the LD-50 dose is a reliable estimate there appear to be conditions in the development which cause the early cleavage stage to be very sensitive, cause the late cleavage (18 t. u.) to the eyed stages (332 t. u.) to reach a plateau of sensitivity of between 400 and 500 r, and cause the late eyed stages to again decrease rapidly in sensitivity. Similar variations in rate of decrease in sensitivity were seen in the chicken embryo by Wilson (1935).

Figure 8 and Table 3 indicate four fairly definite periods of high mortality in each of the groups as follows: one reaching a peak at about 8 days (ca. 160 t. u.) during the late cleavage stages; one reaching a peak at about 22 days (ca. 475 t. u.) apparently during development of the eye, brain and caudal region; a rapid increase in mortality at about 31 days (ca. 709 t. u.) during the early larval stages; and one increasing slightly at about 50 days (ca. 1277 t. u.) during the period of first active feeding. There was obvious variation from group to group and from dose to dose in the appearance of the periods, the heavier doses bringing on the period of high mortality somewhat earlier than lower doses; also there was apparently some variation in the eggs from different parents. The third and fourth periods of high mortality appear to be similar to those obtained by X-irradiation of chinook salmon in the eyed stages (Welander et al., 1948). Corbella (1930) noted somewhat similar periods in experiments with X-rayed trout embryos.

The nature of the trends previous to irradiation in the early eye stage (Figure 8) indicate that handling of the eggs during X-irradiation of this stage caused subsequent mortalities in all but one of the lots. Experiences of other investigators indicate that certain stages are more susceptible to physical damage than others, and this will be discussed later. Mortality rates in the eggs previous to irradiation are indicated in the graphs by the extension of the line below the abscissa.

The periods of maximum mortalities do not, in most cases correspond to those obtained in fish embryos by sublethal temperatures, asphyxial H_2, KCN (Vernídub, 1939), vibrations (Hata, 1927 and 1929), concussion (Rollefsen, 1932), electric shock (Scheminzky,

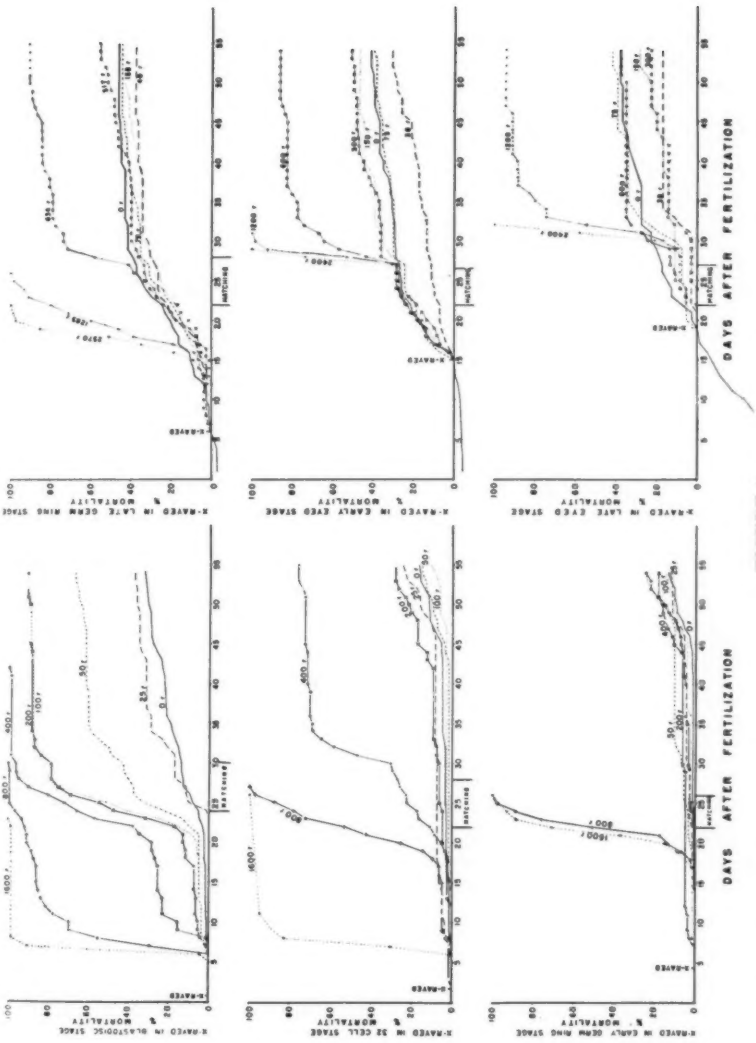

FIGURE 8

Relation of cumulative mortalities to days after fertilization, days after irradiation, and dose. Mortalities before irradiation are indicated in the three later stages by solid lines below the abscissa.

TABLE 3
Approximate stages of development at which the death rate was high compared with dose, time, and temperature units.

Stage irradiated	Dose in r	Approximate stages of development at which death rate was high	Time in days	Temperature units
One-cell	1600	Late cleavage	6-8	119-162
	800	Late cleavage	6-9	119-182
	400	Early germ ring; early eye to hatching	8-11;20-28	162-222;427-571
	200	Early eye pigmentation through hatching	22-28	475-571
	100	Early eye pigmentation through hatching	22-28	475-571
	50	Early eye pigmentation; early larvae	21-26;30-34	451-571;680-799
	25	Hatching; early larval stage	24-28;31-34	520-624;709-799
Thirty-two cell	1600	Late cleavage	6-8	
	800	Early eye pigmentation to hatching	18-26	383-571
	400	Early eye pigmentation; early larvae	21-25;31-34	451-545;709-799
	200-0	End of yolk	44-53	1104-1366
Early germ ring	1600	Early eye pigmentation	19-26	404-571
	800	Early eye pigmentation	19-26	404-571
	400-0	End of yolk	45-53	1134-1366
Late germ ring	2570	Early eye stage or caudal formation	15-20	332-452
	1285	Early eye pigmentation to hatching	16-23	356-532
	634	Early eye pigmentation through hatching	17-29	378-711
	317-0	Early eye pigmentation through hatching	16-29	356-711
Early eye	2400	Caudal formation; early larvae	16-24;27-29	356-561;650-711
	1200	Caudal formation; early larvae	16-24;27-30	356-561;650-737
	600	Caudal formation; early larvae	16-24;27-33	356-561;650-819
	300-0	Caudal formation	16-24	356-561
Late eye	2400	Early larvae	29-32	651-739
	1200	Early larvae	29-37	651-884
	600-0	Early larvae	30-34	680-799

1922), and high temperatures (Trifonova, 1939). These physical and chemical agents usually produced maximum rates of mortality during closure of the blastopore (Hayes and Armstrong, 1942) and some increase in rate during gastrulation, cleavage, and just before hatching. It is only the latter period that corresponds to any of those noted in X-irradiated trout.

Lack of correspondence of the periods of high death rate from X-rays and from other factors might be due in part to the time lapse between administration of dose and effect in the irradiated embryos. At present there is not enough data on the various stages of development of salmonoids to complete the story of critical stages following irradiation.

Growth

Averages of lengths and weights of survivors indicate a differential rate of growth varying with dose and with stage of development. The standard length was used as the most accurate measure of body length (from the tip of the snout to the base of the caudal fin), and the data are recorded in Table 4 and in Figure 9. Average weights were obtained by taking the total weight of a lot of fish and dividing by the number of specimens. It will be noted that the averages of the weights and of lengths follow the same trends and we may assume that the standard deviations, standard errors and the range are probably similar (Table 4).

Figure 9 is a graph of the range (vertical line), the mean (short horizontal line), two standard errors of the mean on either side of the mean (dark bar), and one standard deviation on either side of the mean (open bar). The method is used for a comprehensive analysis and a rapid and economical comparison of a series of samples (Hubbs and Hubbs, 1953). A broad overlap of the dark bars indicates little or no difference between two samples whereas any considerable separation of these bars indicates a very great and significant difference. In a number of cases, of border-line significance, the t-test was calculated and P, the probability that the differences were not significant, is noted in the text.

Differences between control lots of the various stages are attributable mainly to eggs obtained from different females, as noted in a previous section of this paper, and to varying sensitivity of the stages to handling during irradiation. For example, eggs handled during the

TABLE 4
Relationship of standard length in millimeters and of weight in grams to dose at various stages of development.

Stage of development								
One-cell	Dose	0r	25r	50r	100r	200r		
	Length $\bar{x}$	25.2	24.9	24.6	25.5	25.0		
	$s_{\bar{x}}$	1.26	1.73	1.83	1.14	2.19		
	Weight $\bar{x}$	0.21	0.21	0.21	0.24	0.22		
Thirty-two cell	Dose	0r	25r	50r	100r	200r	400r	
	Length $\bar{x}$	25.5	25.2	25.9	25.0	25.0	22.8	
	$s_{\bar{x}}$	1.95	1.62	2.17	1.05	1.88	2.78	
	Weight $\bar{x}$	0.23	0.22	0.25	0.23	0.23	0.19	
Early germ ring	Dose	0r	25r	50r	100r	200r	400r	
	Length $\bar{x}$	26.1	27.5	25.7	25.7	25.4	25.4	
	$s_{\bar{x}}$	2.37	2.12	2.28	1.70	1.99	1.87	
	Weight $\bar{x}$	0.26	0.31	0.25	0.26	0.24	0.23	
Late germ ring	Dose	0r	40r	79r	158r	317r	634r	
	Length $\bar{x}$	27.9	26.8	28.0	27.4	27.4	22.1	
	$s_{\bar{x}}$	2.23	2.38	2.35	2.39	3.01	1.74	
	Weight $\bar{x}$	0.31	0.28	0.33	0.31	0.33	0.16	
Early eye	Dose	0r	38r	75r	150r	300r	600r	
	Length $\bar{x}$	25.7	24.7	24.4	24.7	24.4	18.6	
	$s_{\bar{x}}$	2.27	2.11	2.14	1.81	2.19	2.27	
	Weight $\bar{x}$	0.24	0.22	0.23	0.24	0.22	0.09	
Late eye	Dose	0r	38r	75r	150r	300r	600r	1200r
	Length $\bar{x}$	26.2	26.2	25.6	26.6	25.7	25.4	19.2
	$s_{\bar{x}}$	2.33	1.31	2.76	1.77	1.61	1.73	0.78
	Weight $\bar{x}$	0.28	0.27	0.26	0.29	0.27	0.24	0.08

germ ring stages are much more susceptible to injury than those handled during the cleavage or the eyed stages.

Growth in length appears to be retarded after doses administered in the early eyed stage, at a time when the caudal region is growing fairly rapidly. Growth was not affected in survivors irradiated during the one-celled stage, and was but little affected in fish surviving most doses during the 32-cell or germ ring stage, and pre-hatching stage. Doses of 200 r or more had some effect on the early germ ring stage ($P = .06$); 634 r affected the growth of fish after irradiation in late germ ring stage and 1200 r was effective in a very small sample of survivors at pre-hatching. Doses of 400 r inhibited growth of fish irradiated in the 32-cell stage.

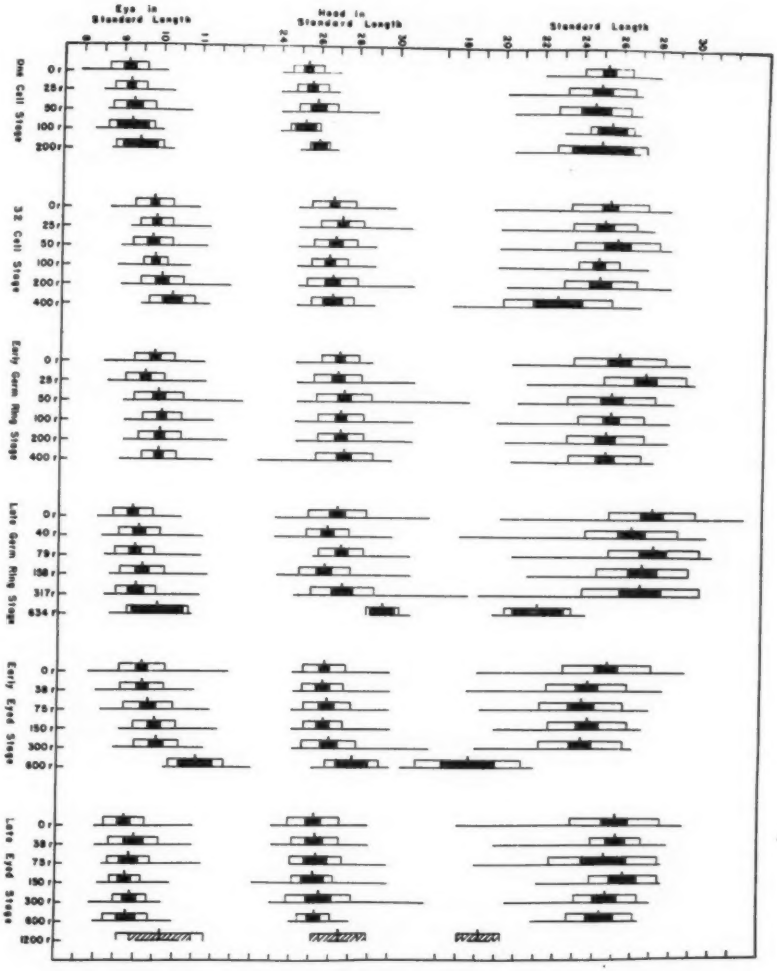

FIGURE 9

Graphical analysis of the resulting relationship of body length (in mm.), head and eye (in per cent of body length), of trout to dose and stage of development at the time of irradiation. The range is shown by the long vertical line; the mean by the short horizontal bar at right angle to the range; four standard errors of the mean by the black bar; and one standard deviation on either side of the mean, by one-half of a black bar plus the white bar.

One of the disturbing features of the data in the effects on growth and on other characters is that significant differences appear inconsistently and not in line with the general trend, at comparatively low doses, i.e., at 25 r after irradiation in the early germ ring stage. It would appear that this might represent a stimulation of growth by comparatively low doses. Stimulation has been reported intermittently in the literature (Brunst, 1952), and may explain these variations. However, it will be noted that none of the means has a smooth trend and some of the variations can be attributed in part to small samples and in part to experimental error.

The effects on growth are similar to results obtained in other experiments on fishes (Welander et al., 1948; Solberg, 1938; Foster et al., 1949) as well as other vertebrates (Rugh, 1954; Wilson, 1954). The results are also similar to effects on fish of high temperatures (Merriman, 1935) of light (Smith, 1916; Willer, 1928), and of increased atmospheric pressure (Goff, 1940).

Apparently all of the physical factors mentioned above have an inhibiting or retarding influence on growth. It appears that the most actively proliferating tissue is the most sensitive for ionizing radiations (Rugh, 1949), the effects being greatest on the dividing cells. Apparently the rate of cell division is reduced mainly by increasing the intervals between divisions (Giese, 1947). Solberg (1938) found that changes in sensitivity during the early development of the *Fundulus* embryo paralleled the changes in the mitotic index.

In spite of retardation of growth, hatching time in this experiment was not greatly altered by irradiation. The time of hatching and the length of the hatching period from the first to the last egg to hatch were not affected. Only those eggs irradiated with high doses during the one-cell, 32-cell, early germ ring and late germ ring stages failed to hatch. Large numbers of eggs hatched after irradiation of 2400 r in early and late eyed stages, indicating that the hatching enzyme (Hayes, 1949) was probably not affected.

Corbella (1930) found that the hatching period was prolonged by increasing doses of X-rays in trout and Hinrichs (1925) observed a similar delay in hatching in *Fundulus heteroclitus*. Further differences from the present experiment are noted in the reactions of trout eggs to temperature and light in which the hatching time is shortened by increased temperatures (Merriman, 1935), and increased light (Page, 1885; Smith, 1916).

Body Proportions

The relative size of the head and eye was studied in surviving fish by dividing the character into the standard length. The data are recorded in Figure 9 in per cent.

The graphs of the head length usually revealed only very slight but inconsistent trends toward larger head ratios, relative to length, with increased dose. No trend was observed in fish irradiated during the 32-cell, early germ ring and late eyed stages. Doses of about 600 r caused significant differences in head proportions after irradiation of eggs in the late germ ring stage and in the early eyed stage, periods when the head and body are growing and differentiating rapidly. The trend toward production of larger head ratios with increased dose administered during the one-cell stage is of interest but is erratic and must await confirmation by further experimentation. Generally, the earlier the stage the greater and more general are the effects (Handford, 1949). This might indicate that the size of the target in the one-cell egg and the stage of development both argue for an "all or none" effect in which surviving fish would be expected to escape any effects.

Pigmentation

Pigmentation, as measured by the number of parr marks large enough to cross the lateral line, was affected significantly at every stage of development and by most dosages used. In the majority of the lots the trends are well marked (Figure 10). X-irradiation as low as 25 r caused significant differences in parr mark numbers administered in 32-cell and early germ ring stages ($P < .04$); 50 r in the one-cell stage; 79 r in the late germ ring stage; 150 r in early eyed stage ($P < .02$) and 300 r in pre-hatching. Trends of the means are not consistent, if correlated with dose, in the 32-cell and early germ ring stages.

Color changes have been noted in birds after irradiation (Cole and Finley, 1942) and in mice (Russell and Major, 1952) but in both the effect was essentially an increase or production of white spots on the body. Rugh (1949) noted that chromatophore inhibition was a surface indicator of severe radiation damage in frog embryos. These effects do not seem comparable to the reduction in the number of parr marks as seen in the trout. The reduction appears to be related to the general effect of retardation of and the consequent reduction in meristic characters.

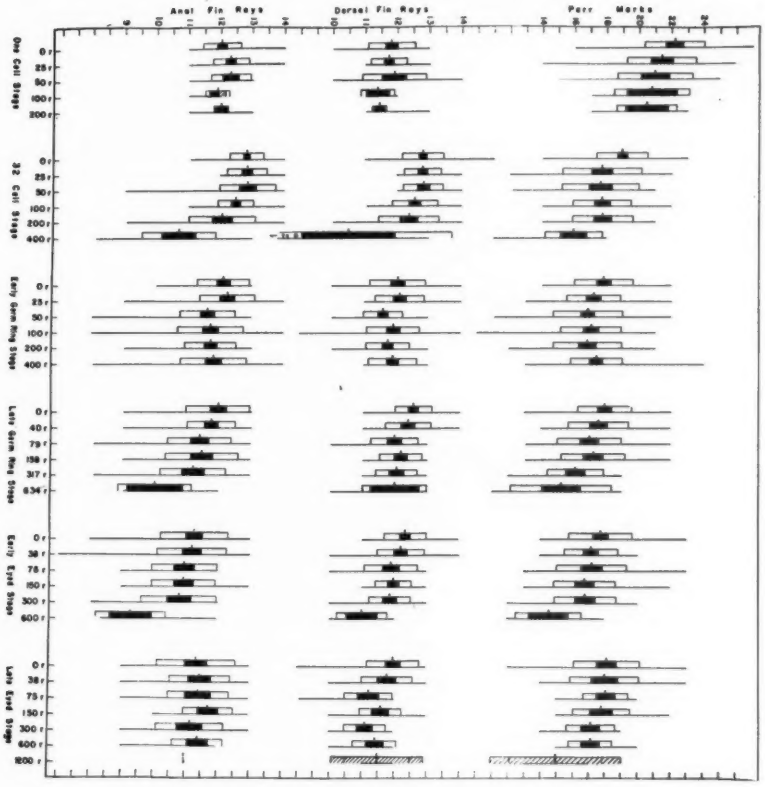

FIGURE 10

Graphical analysis of the relation of the number of parr marks, dorsal and anal fin rays of trout to dose and stage of development at the time of irradiation.

Other factors which have influence on the growth and development of integumentary pigment in fishes are light (Osborn, 1948) and temperature (von Frisch, 1911). With the present evidence it is difficult to compare these factors with that of X-irradiation. Effects on the parr marks from X-irradiation might be more comparable to the effects of such an environmental factor as temperature as indicated by Schmidt (1917) for *Zoarces viviparous,* in which the number of pigment spots in the dorsal fin varied from 7 to 22. He concluded that the variation was in direct relation to the number of vertebrae.

Fins

A reduction in the number of dorsal fin rays was noted (Figure 10) in fish irradiated with 100, 200 and 400 r during the 32-cell stage ($P < .02$); with 100 and 200 r in the one-cell stage ($P > .05$); with 75, 150, 300 and 600 r in the early eyed stage; and with 75, 150, 300 and 600 r in the late eyed stage ($P > .02$).

Similar trends were noted in the numbers of anal rays in fish irradiated with 100, 200 and 400 r in the 32-cell stage; with 50 to 400 r in the early germ ring stage ($P > .05$); with 79 to 634 r in the late germ ring stage; and with 300 and 600 r in the early eyed stage ($P = .02$).

Inconsistencies, amounting to a significant increase in the number of anal fin rays, was noted in fish irradiated with 25 and 50 r during the one-cell stage, and there appears to be a trend in this direction in fish given low doses in the pre-hatching stage. This behavior may result from either experimental error or the "stimulative" effect of low doses. The stimulating effect of low doses of X-rays has been discussed in relation to growth, but it may be added here that in the case of *Drosophila* low doses (155 r) increased the eye facet number six to seven per cent, whereas higher doses decreased the number (Luce, 1951).

The reduction in number of different anatomical characters has also been noted, after irradiation, in mice digits and vertebrae (Russell and Russell, 1954), and in amphibian digits (Handford, 1949, and Rugh, 1950). Solberg (1938) indicates that a reduction of myotomes occurs after X-irradiation of *Fundulus* embryos.

There are a number of other factors which have been found to be effective in altering the number of characters in fishes. Temperatures, within the normal tolerance of the fish, have long been known to affect the development of the number of meristic characters in fishes (Tåning, 1952). Light has been found to be a factor with a definite effect, the brighter the light during the development the greater the reduction in the number of vertebrae in the Grunion (McHugh, 1954) and in the Cod (Dannevig, 1932). Combinations of salinity and temperature affected the number of fin rays in Sticklebacks (Heuts, 1949) and increased atmospheric pressure during development reduced the number of somites in the Zebra fish (Goff, 1940).

All of these factors, including irradiation, acted similarly in that an

increase in "dose" usually reduced the number of meristic characters. In addition, all of the factors usually acted to retard development. However, not all of the factors act simultaneously to retard development and reduce the number of characters as is the case in X-irradiation. High temperature or increased light, in contrast to X-rays, usually reduce the number of meristic characters, such as fin rays and vertebrae, while increasing the rate of development. This would indicate that basically the effects are different and that light or temperature does not affect the dividing cell in the same manner as X-irradiation.

Anomalies

In this preliminary experiment only the visible structures in the surviving fish such as fins, eyes and body proportions were examined for abnormalities. Generally speaking, most of the anomalies found were common to both the control and the irradiated fish (see Table 5). These anomalies consisted mainly of microphthalmia, abdominal and caudal foreshortening or abdominal lordosis (Figure 11). In the eggs from one female (32-cell and early germ ring stages) a distortion of the hyoid apparatus was peculiar and consistent through all the lots.

In at least one group an anomaly apparently peculiar to increased radiation was noted. Fish irradiated with 200 and 400 r at the 32-cell stage had abnormal dorsal and adipose fins, these being absent in some, split or otherwise anomalous in others (Figure 11).

Increase in abnormalities with dose definitely occurred in fish irradiated at the 32-cell and early eyed stages. There appears to have been some increase in trout irradiated in the one-cell and 2/3rds epiboly stages but these results are obscured by the small samples involved in the former and by large numbers of abnormalities in the latter. No significant rise in abnormalities was apparent in the two other groups, both of which had large numbers of abnormal fish in the controls.

The production of abnormalities by X-irradiation of developing embryos has long been known and has reached a point where it is used not only to analyze the effects of irradiation but also to analyze the developmental processes. Irradiation with X-rays has revealed, to a certain degree, critical stages in the development of the embryo and, in some cases, critical stages in the development of certain organs and tissues (Solberg, 1938; Russell and Russell, 1954). Comparison

TABLE 5

Number of superficial abnormalities compared by structure, by dose, by egg lot and by number of fish in surviving trout.

Stage irradiated	Dose	Dorsal fin	Adipose fin	Caudal fin	Anal fin	Ventral fin	Pectoral fins	Micophthalmia	Hyoid region	Abdominal region	Caudal region	Total number of fish	Total number of anomalies	Number of fish w/one or more anomalies	Per cent abnormal fish
One-cell	0r	3	1	2	2	6	1	2		4	3	181	24	8	4.4
	25r			1	1	1	1			1		54	5	2	3.7
	50r				1			1			1	27	3	2	7.4
	100r							2				8	2	2	25.0
	200r							2				9	2	2	22.0
Thirty-two cell	0r							1	7	1		120	9	9	7.5
	25r								1		2	61	3	3	4.9
	50r								3	1	1	45	5	5	11.1
	100r							1	6	1		59	8	8	13.5
	200r	2	8		1				3	2	3	46	19	18	39.2
	400r	6	13	1	1			3	1	3	1	20	29	18	90.0
Early germ ring	0r		3						23	1	3	142	30	27	19.0
	25r								8		1	61	9	9	14.8
	50r								15	2	3	66	20	18	27.3
	100r								5	1	1	73	7	7	9.6
	200r								7	1	6	66	14	13	19.7
	400r				1				10	1	3	65	15	13	20.0
Late germ ring	0r							4		8	7	83	19	13	15.7
	40r							2		5	1	49	8	8	16.3
	79r			1				4		4		48	9	7	14.6
	158r							1		4	1	45	6	5	11.1
	317r							6		3	2	34	11	9	26.5
	634r		1					1				7	2	2	28.6
Early eye	0r							9		11	4	92	24	18	19.3
	38r							7		4	3	56	14	10	17.9
	75r							7		11	3	42	21	13	31.0
	150r							5		7	2	43	14	11	25.6
	300r							6		9	2	39	17	14	35.9
	600r							3		7	2	11	12	7	63.7
Late eye	0r							9		8	2	49	19	16	32.7
	38r							4		2	3	29	9	7	24.1
	75r							3		2	3	22	8	6	27.2
	150r							5			2	25	7	5	20.0
	300r							6		2	1	27	9	6	22.2
	600r							3		3	2	23	8	6	26.1
	1200r											2	0	0	0

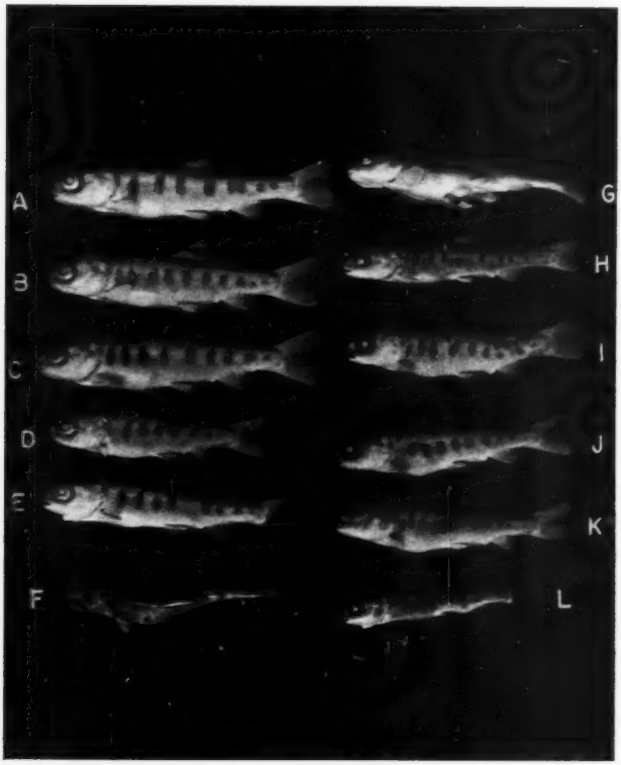

FIGURE 11

Protograph of surviving trout which show some of the prevalent types of abnormalities compared with nórmal (A). Loss or modification of the dorsal fin is illustrated by specimens C, I, J, K; loss or modification of the adipose fin by B, I, J, and K; reduction of the anal fin by specimens D, J, and K; modified ventrals by G; eye abnormalities by H and K; modified hyoid region by E and L; shortened or twisted abdominal region by F and J; and shortened caudal region by D. Specimens A. to F inclusive were irradiated with 200 r during the 32-cell stage, and specimens G to L inclusive were irradiated with 400 r during the 32-cell stage (X 2.4).

of critical stages of fish as revealed by X-irradiation with critical stages noted in other vertebrates is not possible with present knowledge although the abnormalities produced are, in many ways, similar (i.e., microphthalmia). Similarities are further made evident in that the most cases show early developmental sensitivities which decrease in the older embryos.

Resemblances of the present experiment to X-irradiation experiments on fish by other workers are not clear because of differences in experimental techniques (i.e., dose) and differences in analysis. Two experiments, one on embryos of *Fundulus heteroclitus* (Solberg, 1938) and one on developing trout and perch (Corbella, 1930) have certain features in common with each other and with the present experiment. In *Fundulus* the dose required to deform the embryo at 108 hours (roughly comparable to the early eyed stage in trout) is about ten times that required to deform the *Fundulus* embryo during cleavage. In the trout, if we consider mortality as due to some abnormality, the dose required to deform the embryo in the early eyed stage is about seven times that required for the one-cell stage. Solberg also noted that, in *Fundulus* embryos, the order of sensitivity bears little or no relation to the order in which the organ develops. This is substantiated by the effect of X-rays on the adipose and dorsal fins of trout during the 32-cell stage, both being disturbed long before the anlagen of either character appears. This very probably happens to other organs, some of which are vital to survival and might be revealed in an examination of the mortalities. Anomalies arising from irradiation of cleavage stages would appear to result from a disturbance of the precursors.

Sensitive critical periods as revealed in the study of *Fundulus* embryos are somewhat similar to those of trout, the cleavage stages being the most sensitive. Another sensitive period was noted during the early eyed stage with the germ ring stages being more or less resistant.

Corbella also found that the segmentation stages were more radiosensitive than the eyed embryo or the completed embryo (just before hatching). Apparently he noted effects to doses as low as 97 r, with doses of 440 r causing appreciable mortality in trout. He also noted the increase in certain abnormalities with dose, such as microphthalmia, curved embryos and embryos in which the body parts were not in proportion to one another.

General Conclusions and Discussion

An examination of the foregoing data indicates that the most radiosensitive stage as determined by the LD-50 dose (58 r) is the one-cell or blastodisc stage. According to comparative figures the one-cell stage, about three hours after fertilization, is five to fifteen times as radiosensitive as any of the other five stages investigated.

Investigators are in general agreement that the early stages (segmentation) are the most sensitive although this hypothesis may be open to some question when compared with some of the results obtained with trout embryos, chick embryos and mice embryos.

If, for example, we consider only the surviving individuals and measure the effects of the various doses of X-ray by comparing the irradiated survivors with the controls, we find that in terms of dose and effect the pattern is somewhat different. For example, a significant retardation of growth was noted from 38 r after irradiating embryos in the early eyed stage, and 400 r was effective after irradiating the 32-cell stage.

In assessing the effects on two body proportions no general conclusions were possible in data pertaining to the head-length and body-length proportions. All stages were significantly different only at comparatively high doses. The trend toward a proportionately larger eye was marked and the eye ratio was significantly greater after irradiation with 150 r in the early eyed stage, but apparently was affected by 200 r in the 32-cell stage, long before the appearance of the anlage of the eye.

Color, as manifested by the number of large parr marks present, was affected at every stage irradiated, often significantly by comparatively low doses. At the 32-cell stage, and at the early germ ring stage, 25 r significantly reduced the number of parr marks. In the one-cell stage, 200 r was effective, and 300 r was effective in other stages. Of all the characters investigated, the color pattern was apparently the most generally sensitive to X-rays; however, there are inconsistencies in the trends of the means in this, as well as in many of the other characters, which only further experiments can resolve.

The anal and dorsal fins were differentially affected, that is, irradiation at any one stage did not always alter these characters at the same rate. The number of dorsal fin rays was significantly reduced by 75 r administered during the early eye stage and the late eye stage. One hundred roentgens apparently reduced the dorsal rays in the one-cell and 32-cell stage; and 79 r was effective during the late germ ring stage. No effects on the dorsal fin were noted in the early germ ring stage at the highest doses, but the anal fin appeared to be sensitive during this stage, 50 r producing measurable effects. Irradiation of 79 r in late germ ring stages significantly reduced the number of anal fin rays and 100 r was effective when given during the 32-cell stage.

Both the dorsal and the anal fins were sensitive to X-rays during a considerable range of developmental stages, the dorsal fin during the 32-cell and early eyed stages, the anal fin during the 32-cell, late germ ring and early eyed stages.

An examination of surviving fish for abnormalities revealed a percentage increase with dose after irradiation in the one-cell, 32-cell, and early eyed stages. These abnormalities were of the same types as found in the controls except for those involving the adipose and dorsal fin after irradiation in the 32-cell stage by 200 and 400 r.

An attempt to summarize the effects of X-irradiation was made using the methods described by Clark (1952) as a coefficient of divergence for use with multiple characters. Characters used in the calculations were standard length, head in standard length, eye in standard length, parr marks, dorsal rays and anal rays. In the calculations, each irradiated group was compared with the controls and the results are noted in Figure 12.

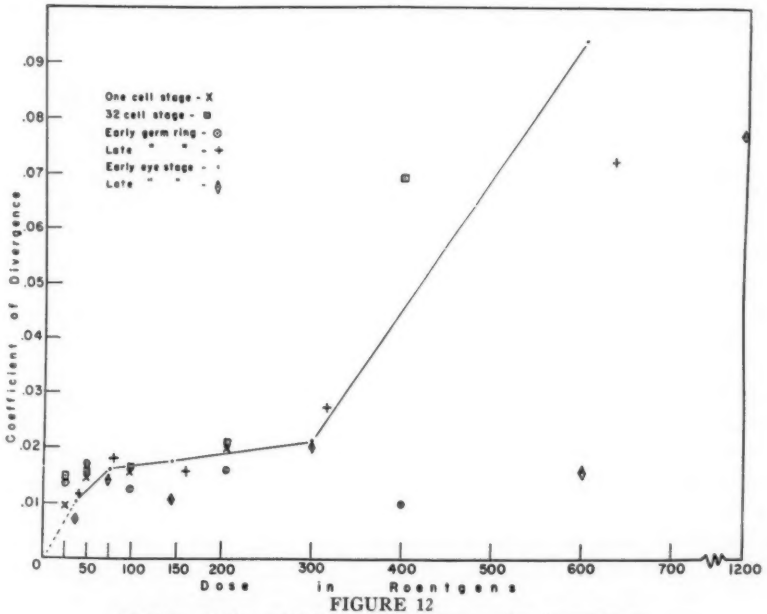

FIGURE 12
Relation of the coefficient of divergence to dose in roentgens.

In Figure 12 the direct correlation of dose with increase in divergence from the control fish is obvious. The variation in the data is great and may be in part due to the differential sensitivity of the stages irradiated. The fish irradiated during the pre-hatching stage generally had a lower coefficient of divergence at most doses than the other stages. Trout irradiated with 400 r in the early germ ring stage (1/8th epiboly) had a comparatively low coefficient which was not within the trends shown by the other groups. Both of these stages were consistently more resistant to X-irradiation than the other four stages in the experiment.

Throughout this paper comparisons have frequently been made of the effects of X-irradiation and those of temperature, light and other factors on the development of fishes. Similarities were pointed out wherein all these factors caused retardation of development, affected the number of meristic characters, and to a certain extent, caused similar anomalies. In retarding development all the factors probably acted to increase the intervals between cell divisions. Absence and general disturbances of the adipose and dorsal fin in otherwise normal appearing fish indicated a disturbance of the precursors at the 32-cell stage. The effects of X-irradiation also differed in that the retardation of development usually accompanied a reduction in the number of fin rays, whereas the effects of temperature, light, salinity, etc., combined retardation of development with an increase in the number of meristic characters. Further, and contrary to results obtained by other investigators, hatching appeared not to be affected by the X-ray doses used. Temperature, light and a number of other factors drastically alter hatching time. This is tentatively interpreted as lack of effect on the hatching enzyme by comparatively low doses of X-rays.

The question of a "stimulating" effect on animals by X-irradiation is far from settled. The inconclusive evidence from the literature and the evidence from this experiment indicate that further careful experimentation on developing embryos with low doses is needed.

From the foregoing remarks and from a survey of the data it appears that the early germ ring stage is generally the least sensitive of the six stages investigated. On the other hand, the most sensitive stage is not easy to define if we measure sensitivity by the doses required to produce significant effects. In both the 32-cell stage and early eye stage most of the characters are considerably altered by comparatively low doses, which are as low as or lower than doses

which killed 50 per cent of the embryos in the one-cell stage. The late germ ring stage also appears very radiosensitive judging from the trends of the means and the doses at which the means of the irradiated trout become significantly different from the controls. The two remaining stages, the one-cell and the late eyed stage, seem much less sensitive than the others if we disregard mortalities in the former.

A review of the literature (Needham, 1931; Hyman, 1921; Vernidub, 1939) shows that a large variety of agents and conditions produce similar defects in fish embryos. These defects are concerned with practically all parts of the embryo and if the part is vital the defect results in early death. The periodicity noted in the rates of mortalities in trout may indicate that the failure of certain vital structures or processes to develop terminates the development of the embryo.

The results definitely indicated that critical periods, during which the radiosensitivity of certain organs is comparatively high, may extend over a long period of development. Further, the critical period may begin long before the anlagen of an organ appears.

Hyman (1921) and others explain teratological development as defects due to the differential susceptibility of the embryo, with its various parts, to the agent. The differential susceptibility has been correlated with metabolic conditions, in the eggs and embryos and to the differentiation of the embryo, two processes which may not occur simultaneously (Jones, 1939).

Many investigators (Evans, 1936; Packard, 1935; Rugh, 1949) correlate radiosensitivity with metabolic rate of the dividing cell. Others (Hicks, 1953; Russell and Russell, 1954) correlated sensitivity with differentiation of the embryo.

Correlation of sensitivity by use of periods of heavy mortalities of trout with oxygen consumption (Vernidub, 1939) was sufficiently marked to indicate that metabolic rates were concerned. Correlation using LD-50 doses of X-rays on trout with growth rate data and glycolosis data of Vernidub appears significant, again indicating that the critical periods coincide. Vernidub's analysis indicates that critical periods due to agents that cause asphyxiation or due to high temperatures occur during periods when the intensity of differentiation rises. These periods do not always correspond to those sensitive periods noted in trout after irradiation, although this does not mean that no effects were obtained during differentiation of the trout embryo by X-rays. More research is needed to determine the roles played by mitosis and differentiation after irradiation of fishes.

The periods of high rates of mortality in trout compare with similar experiments by Corbella (1930) after irradiation of similar stages of development. Corbella found that trout segmentation and early eyed stages were sensitive and that high rates of mortality occurred in late segmentation, late germ ring, early eyed, pre-hatching and post-hatching stages. Irradiation of eyed stages of chinook salmon (Welander et al., 1948) evoked similar effects to those obtained in trout embryos in eyed stages. Solberg's (1938) work on *Fundulus* was not in as close agreement, segmentation and gastrulation appearing to be sensitive stages. Differences in results might be due to differences in reaction of different species of fishes as well as to differences in dose and interpretation of results. Gastrulation was found to be a radiosensitive stage in other animals, namely, in *Daphnia,* in *Strongylocentrotus* in *Ascaris,* and in Amphibia (Needham, 1931).

The production of phenocopies by irradiation has been noted in many animals and is now tentatively established in fishes. The effects on fish are similar to those produced by temperature, light and other factors which apparently interfere with developmental processes. Tåning's (1952) review and investigation on trout has served to delineate some of the critical stages at which vertebrae and other characters might be altered by temperature. The results of the investigation of X-irradiation of trout suggests that X-rays might serve as a much better tool to use for analysis of the phenocritical periods of certain structures and organs, especially those structures (i.e., fin rays, color) which form the basis for the separation of races, subspecies and even species of fishes.

Summary

1. The results of experiments are presented which involved the exposure of six different embryonic stages of rainbow trout, ranging from one cell to hatching, to graded doses of X-rays, from 25 to 2570 r.

2. Mortalities indicated high sensitivities during the one-cell stages with an $L.D._{50}$ of 58 r at the time the yolk was absorbed. Doses of 300 r to 900 r caused $L.D._{50}$ in the other five stages, with an irregular relation of decreasing sensitivity with age of embryo.

3. A rather uniform periodicity in mortality rates was evident in comparisons of all the mortality data, suggesting that irradiation interfered with some precursors which were not required for survival until a definite, critical stage of development was reached.

4. Retardation in growth of length and weight was significant after doses as low as 38 r were administered during the early eyed stage, at the time of the formation of the caudal structures. Apparently, 200 r or more was required to interfere with growth in the other embryonic stages.

5. Some evidence is presented which indicates a tendency for retarded embryos to preserve a more juvenile appearance than the controls, viz., a larger eye and head in proportion to the size of the body. This was most noticeable in embryos irradiated during the 32-cell, late germ ring, and early eyed stages.

6. The number of parr marks was usually significantly reduced in all stages after doses of 300 r or more, with doses as low as 25 r significantly altering the number in embryos irradiated during the 32-cell stage.

7. Reduction in the number of dorsal and anal fin rays was general after irradiation of 32-cell, late germ ring, and early eyed embryos. Doses from 75 to 100 r were significantly effective in reducing the fin ray number in these stages.

8. Gross superficial abnormalities observed in X-rayed trout were similar, though usually more numerous, to those found in the controls, with the exception of anomalies of the dorsal and adipose fin produced by 200 and 400 r X-rays of 32-cell embryos.

9. Comparisons of the effect of X-irradiation on trout were made with effects on fishes, noted in the literature, of temperature, light and other physical factors.

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BOOK REVIEWS

Basic Mechanisms in Radiobiology. II. Physical and Chemical Aspects (Proceedings of an informal Conference held at Highland Park, Ill., May 7-9, 1953). Edited by J. L. Magee, M. D. Kamen and R. L. Platzman, National Academy of Sciences—National Research Council publication No. 305, Washington, D. C., 1953. 145 pages.

This is a report of the third conference organized by the Subcommittee on Radiobiology of the Committee on Nuclear Science of the National Research Council. In two previous conferences a concerted effort was made to bring together representatives from the many diverse disciplines who could contribute to advances in the broad field of radiobiology. As is indicated in the title this volume is concerned with the contributions which the physical and chemical sciences can make to our understanding of the basic mechanisms in radiobiology, or more specifically of "the nature of the mechanisms involved in the early stages of the interaction of radiation and biological systems".

The volume is composed of five chapters each of which centers around a specific topic which was first reviewed by a chosen speaker. The first two chapters presented by R. L. Platzman deal with energy transfer from incident radiation and from secondary electrons to matter. The mechanisms of energy degradation and chemical change were considered in the third and fourth chapters. In these, the effects of secondary electrons and of electronic excitation are ably presented by J. L. Magee and H. Linschitz. The last chapter by M. Kamen is a summary dealing with the importance of radiation chemical effects in radiobiology.

The sessions were characterized by comments and questions by all participants both during the main presentation and after it. A fortunate result of the informality was the fact that many of the participants speculated more freely than is usually done in their more formal contributions. The result is a veritable goldmine of ideas, hypotheses and suggested explanations but these in turn led to the regrettable request (in the preface) "that no material in this report should be referred to without the specific permission of or consultation with the authors quoted." It is hoped that future publications under the sponsorship of the National Research Council will not be so handicapped.

In the opinion of the reviewer this volume is very much worthwhile

for those doing advanced work in the field but the beginner will find it somewhat difficult. The volume is a valuable contribution in the field of radiobiology and is recommended to those interested in the basic mechanisms of radiobiological effects.

ARNOLD H. SPARROW

The Microtomist's Formulary and Guide. Peter Gray. Blakiston, New York, 1954. xiii plus 794 pp. Illus. $10.50.

This very extensive and comprehensive compilation has a new format and organization which makes for succinctness and has enabled the author to compress a vast amount of information on every page. The book has been in my hands for several months, but I have delayed preparing the review until it could be compared through actual daily use with other compendia of technical methods. There are many features which are extremely useful although we have found it somewhat difficult to become accustomed to the decimal system, particularly in the matter of bibliographic references. However, the necessity for consulting code numbers is more than compensated for by the completeness of the record in a very short space.

The material is organized in two parts, with a total of 28 chapters. The first part deals with the art of making microscopic slides under the following chapter titles: Dry Wholemounts; Fluid Wholemounts—Aqueous Type; Fluid Wholemounts—Non-aqueous Type; Wholemounts in Gum Media; Wholemounts in Jelly Media; Wholemounts in Resinous Media; Smear Preparations from Fluid Material; Smear Preparations from Cut Surfaces; Squash Preparations from Solid Bodies; Ground Sections; Sections of Free Material; Paraffin Sections; Nitrocellulose Sections; Sections from Double Embedded Material; Frozen Sections; and Injections. The second part is devoted to methods and formulas under the following chapter titles: Preservatives; Fixatives; Accessory Fixative Formulas; Formulas and Techniques for Dye Stains of General Applications; Formulas and Techniques for Dye Stains of Special Applications; Accessory Dye Staining Formulas; Formulas and Techniques for Metal Stains; Accessory Metal Staining Formulas; Solvents and Oils; Mounting Media; Embedding Media; and Various Formulas.

In daily laboratory practice we have found this a very complete and useful addition to our source of technical methods and we believe that it should be extremely valuable to all those who are working in the field of microscopy.

IRENE COREY DILLER

The Antiseptic, Golden Jubilee Issue. Madras, India. April, 1954.

This imposing volume comprising over 700 pages celebrates the Fiftieth Aniversary of *The Antiseptic*, the Indian Journal of Medicine and Surgery founded by Dr. U. Rama Rau in 1904. Medical men and others all over the world have contributed articles to this issue which deals generally with 50 years of progress in medicine and its various branches. So many papers were contributed that they could not all be published in the one issue and will continue to appear through the year in subsequent issues.

These articles deal with medicine in general, with tropical medicine, with nutrition research, with diagnosis and therapeutic aids, with advances in anasthesia, with surgery, psychotherapy, neurology, obstetrics and gynecology, dermatology, ophthalmology, endocrinology and various branches of these disciplines. There is also an article on the use and future of antibiotics; a discussion of the progress being made in the control of tuberculosis; and there are several articles on malignancy and special types of cancer. The diagnosis and treatment of leprosy is also discussed. There appear also several general articles dealing with such subjects as nursing, medical education, medicine and politics, and medical journalism. All of these reflect not only the steady march of medicine throughout the world, but of India's contributions to practically every field of medicine; and depict the vast improvement in medical science in India through the 50 years since the journal was established. To scientists in other parts of the world it comes as welcome news that so much medical progress is being made in India. We wish to congratulate *The Antiseptic* on the obviously important role that it has played in furthering this science in India, and particularly for bringing this accomplishment to the attention of scientists everywhere through the medium of this ambitious volume.

IRENE COREY DILLER